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Glutamine Synthetase (GS)Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: AK0505

Size:100T/48S

Components:

Extract solution: Liquid 60 mL×1. Storage at 4℃ .

Reagent I: Liquid 10 mL×1. Storage at -20℃ .

Reagent II: Liquid 10 mL×1. Storage at -20℃ .

Reagent III: Powder×2. Storage at -20℃ . Add 5 mL of distilled water to each bottle before use. Separate storage the unused reagent and stored at -20℃ .

Reagent IV: Liquid 15 mL×1. Storage at 4℃ .

Product Description:

Glutamine synthetase (GS) mainly exists in plants, is one of the key enzymes of ammonia assimilation in organism, which can catalytic synthesis of glutamine by ammonium ion and glutamic acid. The synthesis of glutamine not only prevents excessive ammonium ions from being toxic to organisms, but glutamine is also the main storage and transport form of ammonia.

GS catalyzes the synthesis of glutamine from ammonium and glutamic acid in the presence of ATP and Mg^{2+} . Glutamine is further converted to gamma-glutamyl hydroxamic acid, which can form a red complex with iron under acidic condition. This complex has a maximum absorption peak at 540 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, 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 the proportion of bacteria or cells (10^4): the volume of Extract solution (mL) is 500- 1000:1. Suggest add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonic to splitting bacteria and cells (placed on ice, ultrasonic power 200W or 20%, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2. Tissue

According to the proportion of tissue weight (g): the volume of Extract solution (mL) is 1:5~10. Suggest

add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 8000×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

3. Serum sample: direct detection.

II. Detection

1) Preheat microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 540 nm, set zero with distilled water.

2) Add the following reagents in 1.5 mL EP tubes:

Reagent	Test tube (T)	Contrast tube (C)
Reagent I (μL)	160	-
Reagent II (μL)	-	160
Reagent III (μL)	70	70
Sample (μL)	70	70
Mix thoroughly and incubate at 37℃(mammal) or 25℃(other species) for 30 minutes.		
Reagent VI (μL)	100	100
Mix thoroughly and stand for 10 minutes. Centrifuge at 5000×g for 10 minutes at room temperature to remove insoluble materials, and take 200 μL of supernatant to detect the absorbance at 540 nm, record as A_T and A_c respectively. $\Delta A=A_T - A_c$ Each test tube requires a contrast tube.		

II. Calculation:

A . micro glass cuvette

1) Serum (plasma) volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 540 nm changed 0.01 in the reaction system per minute every milliliter of serum(plasma).

GS Activity (U/mL)=ΔA÷0.01×Vrv÷Vs÷T=19×ΔA

2) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 540 nm changed 0.01 in the reaction system per minute every milligram of protein.

GS Activity(U/mg prot)=ΔA÷0.01×Vrv÷(Cpr ×Vs)÷T =19×ΔA÷Cpr

3) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 540 nm changed 0.01 in the reaction system per minute every gram of tissue.

GS Activity(U/g weight)=ΔA÷0.01×Vrv÷(W÷Ve ×Vs)÷T =19×ΔA÷W

4) Bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 540 nm changed 0.01 in the reaction system per minute every 10^4 of bacteria or cells.

GS Activity(U/10^4 cell)=ΔA÷0.01×Vrv÷(500÷Ve ×Vs)÷T =0.038×ΔA

Vrv: Total reaction volume, 400 μL=0.4 mL;

Cpr: Supernatant sample protein concentration (mg/mL);

Ve: Extract solution volume,1 mL;

Vs: Sample volume (mL), 70 μL=0.7 mL;
T: Reaction time (min), 30 minutes;
W: Sample weight, g;
500: The total number of bacteria or cells, 5 million.

B. 96 well UV plate

1) Serum (plasma) volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 540 nm changed 0.005 in the reaction system per minute every milliliter of serum(plasma).

GS Activity (U/mL)=ΔA÷0.005×Vrv÷Vs÷T=38×ΔA

2) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 540 nm changed 0.005 in the reaction system per minute every milligram of protein.

GS Activity(U/mg prot)=ΔA÷0.005×Vrv÷(Cpr ×Vs)÷T =38×ΔA÷Cpr

3) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 540 nm changed 0.005 in the reaction system per minute every gram of tissue.

GS Activity(U/g weight)=ΔA÷0.005×Vrv÷(W÷Ve ×Vs)÷T =38×ΔA÷W

4) Bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 540 nm changed 0.005 in the reaction system per minute every 10^4 of bacteria or cells.

GS Activity(U/10^4 cell)=ΔA÷0.005×Vrv÷(500÷Ve ×Vs)÷T =0.076×ΔA

Vrv: Total reaction volume, 400 μL=0.4 mL;
Cpr: Supernatant sample protein concentration (mg/mL);
Ve: Extract solution volume,1 mL;
Vs: Sample volume (mL), 70 μL=0.7 mL;
T: Reaction time (min), 30 minutes;
W: Sample weight, g;
500: The total number of bacteria or cells, 5 million.

Note:

Reagent I and II may precipitate, which can be used after re suspension, and the supernatant can be taken for determination after reaction.

Recent Product Citations:

[1] Zhao B, Tian M, An Q, et al. Characteristics of a heterotrophic nitrogen removal bacterium and its potential application on treatment of ammonium-rich wastewater[J]. Bioresource technology, 2017, 226: 46-54.

[2] Yilu Su,Zhi Zhou,Xiaopeng Yu. Possible roles of glutamine synthetase in responding to environmental changes in a scleractinian coral. Molecular Biology Reports. September 2018;(IF2. 107)

[3] 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)

[4] 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)

[5] Shan Li,Yonghang Tian,Kun Wu,et al. Modulating plant growth-metabolism coordination for sustainable agriculture. Nature. 2018;(IF43.07)

References:

[1] Haghighat N. Estrogen (17β-Estradiol) enhances glutamine synthetase activity in C6-glioma cells[J]. Neurochemical research, 2005, 30(5): 661-667.

[2] Bressler S L, Ahmed S I. Detection of glutamine synthetase activity in marine phytoplankton: optimization of the biosynthetic assay[J]. Mar. Ecol. Prog. Ser, 1984, 14: 207-217

Related products:

AK0301/AK0300	Nitrate Reductase (NR) Activity Assay Kit
AK0436/AK0435	Glutaminase (GLS) Activity Assay Kit
AK0434/AK0433	Glutamic Acid Dehydrogenase (GDH) Activity Assay Kit
AK0600/AK0599	Glutamate Synthase (GOGAT) Activity Assay Kit