Glutamine Synthetase (GS)Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: BC0915

Size:100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 10 mL×1	-20℃
Reagent II	Liquid 10 mL×1	-20℃
Reagent III	Powder×2	-20℃
Reagent IV	Liquid 15 mL×1	2-8℃

Solution Preparation:

1. Reagent III: Add 5 mL of distilled water to each bottle before use. It can be stored at -20℃ for 4 weeks after sub packaging. Avoid repeated freezing and thawing.

Product Description:

Glutamine synthetase (GS, EC 6.3.1.2) 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/cell ultrasonic crusher, 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, it is suggested that 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, working time 3s,

interval 10s, repeat for 30

times). Centrifuge at 8000 ×g for 10 minutes at 4℃ 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, it is suggested that 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 ℃ and take the supernatant on ice before testing.

3. Serum sample

direct detection (if the solution is turbid, centrifugate the supernatant and then determine).

II. Detection

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

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

Reagent(μL)	Test tube (T)	Contrast tube (C)
Reagent I	160	-
Reagent II	-	160
Reagent III	70	70
Sample	70	70
Mix thoroughly and incubate at 37 ℃(mammal) or 25 ℃ (other species) for 30 minutes.		
Reagent VI	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×Vrv÷Vs÷0.01÷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×Vrv÷ (Cpr ×Vs) ÷0.01÷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×Vrv÷ (W÷Ve ×Vs) ÷0.01÷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×Vrv÷(500÷Ve ×Vs) ÷0.01÷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=0.07 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×Vrv÷Vs÷0.005÷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×Vrv÷(Cpr ×Vs) ÷0.005÷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×Vrv÷(W÷Ve×Vs) ÷0.005÷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×Vrv÷(500÷Ve ×Vs) ÷0.005÷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.

Experimental instances:

1. Take 0.125 g of kidney of mice, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate $\Delta A=A_T-A_C=0.228-0.07=0.158$. The result is calculated according to the sample weight:
   GS Activity (U/g weight)=38×ΔA÷W=48.032 U/g weight.

2. Take 0.110 g of mouse liver, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate $\Delta A=A_T-A_C=0.76-0.067=0.693$. The result is calculated according to the sample weight:
   GS Activity (U/g weight) =38×ΔA÷W=239.4 U/g weight.
3. Take 0.158 g of soybean sprouts, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate $\Delta A=A_T-A_C=0.142-0.089=0.053$. The result is calculated according to the sample weight:
   GS Activity (U/g weight) =38×ΔA÷W=12.747 U/g weight.

Recent Product Citations:

[1] Zhang Y, Li B, Luo P, Xian Y, Xiao R, Wu J. Glutamine synthetase plays an important role in ammonium tolerance of Myriophyllum aquaticum. Sci Total Environ. 2022 Nov 20;848:157596. doi: 10.1016/j.scitotenv.2022.157596. Epub 2022 Jul 27. PMID: 35905951.

[2] Zhuo TX, Wang Z, Song YY, Yan SW, Liu RD, Zhang X, Wang ZQ, Cui J. Characterization of a Novel Glutamine Synthetase From Trichinella spiralis and Its Participation in Larval Acid Resistance, Molting, and Development. Front Cell Dev Biol. 2021 Sep 20;9:729402. doi: 10.3389/fcell.2021.729402. PMID: 34616735; PMCID: PMC8488193.

[3] Ahmad S, Wang GY, Muhammad I, Chi YX, Zeeshan M, Nasar J, Zhou XB. Interactive Effects of Melatonin and Nitrogen Improve Drought Tolerance of Maize Seedlings by Regulating Growth and Physiochemical Attributes. Antioxidants (Basel). 2022 Feb 11;11(2):359. doi: 10.3390/antiox11020359. PMID: 35204247; PMCID: PMC8869313.

[4] Tong D, Zhu Z, Wu J, Li F, Shen J, Cao J, Tang Y, Liu G, Hu L, Shi W. Impacts of ammonia stress on different Pacific whiteleg shrimp Litopenaeus vannamei families and the underlying adaptive mechanisms. Aquat Toxicol. 2023 Jun;259:106549. doi: 10.1016/j.aquatox.2023.106549.

[5] 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.

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

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com

Related products:

BC0080/BC0085	Nitrate Reductase (NR) Activity Assay Kit
BC1450/BC1455	Glutaminase (GLS) Activity Assay Kit
BC1460/BC1465	Glutamic Acid Dehydrogenase (GDH) Activity Assay Kit
BC0070/BC0075	Glutamate Synthase (GOGAT) Activity Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com