Glutamine Synthetase (GS) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC0910

Size: 50T/24S

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 30 mL×1	2-8℃
Reagent I	Liquid 12 mL×1	-20℃
Reagent II	Liquid 12 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) exists mainly 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, water bath, desk centrifuge, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, 1 mL glass cuvette, ice and distilled Water.

Procedure:

I. Sample preparation:

1. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. 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 200 W, working time 3 s, interval 10 s, repeat for 30 times). Centrifuge at 8000×g for 10 minutes at 4 ℃ to 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 ℃ to remove insoluble materials and take the supernatant on ice before testing.

3. Serum (plasma) sample

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

II. Detection

1) Preheat 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 (μL)	Test tube (T)	Contrast tube (C)
Reagent I	400	-
Reagent II	-	400
Reagent III	175	175
Sample	175	175
Mix thoroughly and incubate at 37℃(mammal) or 25℃ (other species) for 30 minutes.		
Reagent VI	250	250
Mix thoroughly and stand for 10 minutes. Centrifuge at 5000×g for 10 minutes at room temperature, take the 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:

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

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

3) 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, 1 mL;

Vs: Sample volume (mL), 0.175 mL;

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

Ve: Extract solution volume,1 mL;

T: Reaction time (min), 30 minutes;

W: Sample weight, g;

500: The total number of bacteria or cells, 5 million.

Experimental example：

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 $\Delta A=A_T-A_C=0.323-0.047=0.276$.

GS Activity (U/g weight)=19×ΔA÷W=41.952 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 $\Delta A=A_T-A_C=1.203-0.036=1.167$.

GS Activity (U/g weight)=19×ΔA÷W=201.57 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 $\Delta A=A_T-A_C=0.198-0.088=0.11$.

GS Activity (U/g weight)=19×ΔA÷W=13.223 U/g weight.

Recent Product Citation：

[1] Wang Y, Bai J, Wen L, Wang W, Zhang L, Liu Z, Liu H. Phytotoxicity of microplastics to the floating plant Spirodela polyrhiza (L.): Plant functional traits and metabolomics. Environ Pollut. 2023 Apr 1;322:121199. doi: 10.1016/j.envpol.2023.121199. Epub 2023 Feb 2. PMID: 36738884.

[2] Liu J, Tong L, Zhang X, Zhang H, Tao B, Gong Q, Zeng R, Song Y. Dynamic nitrogen reallocation in rice plants upon insect herbivory by a generalist lepidopteran pest Spodoptera litura (Fabricius). Plant Cell Environ. 2024 Jan;47(1):294-307. doi: 10.1111/pce.14736. Epub 2023 Oct 16. PMID: 37843127.

[3] Zhang C, Liu H, Wang J, Li Y, Liu D, Ye Y, Huang R, Li S, Chen L, Chen J, Yao M, Ma C. A Key Mutation in Magnesium Chelatase I Subunit Leads to a Chlorophyll-deficient Mutant of Tea (Camellia sinensis). J Exp Bot. 2023 Oct 31:erad430. doi: 10.1093/jxb/erad430. Epub ahead of print.

[4] Feng K, Wang W, Rong J, Liang J, Mi J, Wu Y, Wang Y. Construction of recombinant Pichia pastoris strains for ammonia reduction by the gdhA and glnA regulatory genes in laying hens.

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

Ecotoxicol Environ Saf. 2022 Apr 1;234:113376. doi: 10.1016/j.ecoenv.2022.113376. Epub 2022 Mar 4. PMID: 35255249.

[5] Sun H, Cui H, Zhang J, Kang J, Wang Z, Li M, Yi F, Yang Q, Long R. Gibberellins Inhibit Flavonoid Biosynthesis and Promote Nitrogen Metabolism in Medicago truncatula. Int J Mol Sci. 2021 Aug 27;22(17):9291. doi: 10.3390/ijms22179291. PMID: 34502200; PMCID: PMC8431309.

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:

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