（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K664-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader (560-580 nm)

Detection range：0.25-25 U/L

Elabscience® Glutamine Synthetase (GS) Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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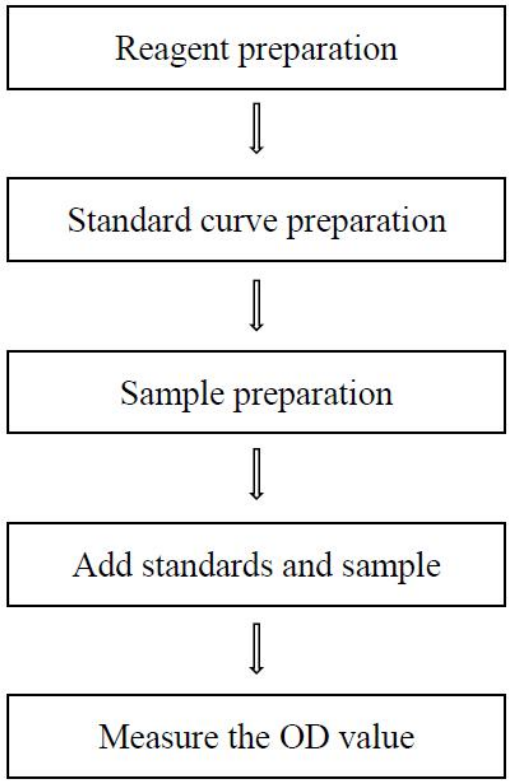
Assay summary



Intended use

This kit can measure glutamine synthetase (GS) activity in serum, plasma and animal tissue samples.

Detection principle

Glutamine synthetase (GS) is one of the central enzymes in nitrogen metabolism With ATP consumption, GS catalyzes glutamate to produce glutamine. The latter is an amino donor of various nitrogenous substances such as proteins, nucleic acids and various coenzymes. As a conditionally essential amino acid for the human body, the production of glutamine has received extensive attention in recent years. GS catalyzes ammonium ion and glutamic acid to synthesize amide, and produces chromogenic substance under the action of invertase and chromogenic agent. The maximum absorption peak is at 570 nm. The activity of GS in samples is calculated by measuring OD value at 570 nm and standard curve.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	40 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Substrate	1.8 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 3 vials	-20°C, 12 months, shading light
Reagent 4	Accelerant	Powder × 3 vials	-20°C, 12 months, shading light
Reagent 5	Catalyst	Powder × 3 vials	-20°C, 12 months, shading light
Reagent 6	5 mmol/L Standard Solution	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	Chromogenic Agent	0.3 mL × 1 vial	-20°C, 12 months, shading light
Reagent 8	Stabilizer	1.8 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (560-580 nm, optimum wavelength: 570 nm), Incubator(37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of enzyme working solution:

Dissolve one vial of enzyme reagent with 200 μL of double distilled water, mix well to dissolve. Store at -20°C for 7 days protected from light.

③ The preparation of accelerant working solution:

Dissolve one vial of accelerant with 0.5 mL of buffer solution, mix well to dissolve. Store at -20°C for 2 days protected from light.

④ The preparation of catalyst working solution:

Dissolve one vial of catalyst with 1 mL of double distilled water, mix well to dissolve. Store at -20°C for 2 days protected from light.

⑤ The preparation of reaction working solution:

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 170 μL of reaction working solution (mix well 145 μL of buffer solution, 5 μL of enzyme working solution, 5 μL of accelerant working solution, 10 μL of catalyst working solution and 5 μL of stabilizer). The reaction working solution should be used up within 4 h.

⑥ The preparation of chromogenic working solution:

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 100 μL of chromogenic working solution (mix well 95 μL of buffer solution and 5 μL of chromogenic agent). The chromogenic working solution should be prepared on spot. Keep it on ice protected from light and used up within 4 h.

⑦ The preparation of 0.5 mmol/L standard solution:

Before testing, please prepare sufficient 0.5 mmol/L standard solution. For example, prepare 1000 μL of 0.5 mmol/L standard solution (mix well 100 μL of 5 mmol/L standard solution and 900 μL of double distilled water). Store at -20°C for 7 days protected from light.

⑧ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.3, 0.4, 0.45, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**	**0.45**	**0.5**
0.5 mmol/L Standard (μL)	0	20	30	40	60	80	90	100
Double distilled water (μL)	100	80	70	60	40	20	10	0

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat kidney tissue homogenate	4-10
10% Rat liver tissue homogenate	5-20
10% Rat spleen tissue homogenate	2-10
10% Rat heart tissue homogenate	2-10
10% Rat lung tissue homogenate	2-10
10% Rat brain tissue homogenate	5-20
10% Mouse liver tissue homogenate	5-20
10% Mouse brain tissue homogenate	2-10
Rat serum	1
Rat plasma	1
Human serum	1
Porcine serum	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

If the measured value of the sample is low, the incubation time can be appropriately extended to 60 min.

Operating steps

① Standard well: Add 20 μL of standard with different concentrations to the wells.

Sample well: Add 20 μL of sample to the wells.

Control well: Add 20 μL of sample to the wells.

② Add 20 μL of substrate to standard and sample wells. Add 20 μL of buffer solution to control wells.

③ Add 150 μL of reaction working solution to each well.

④ Add 40 μL of chromogenic working solution to each well.

⑤ Mix well with microplate reader for 5 s, and incubate at 37°C for 30 min protected from light. Measure the OD value of each well at 570 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum and plasma samples:

Definition: The amount of GS enzyme in 1 L serum or plasma per 1 min that product 1 μmol substrate at 37°C is defined as 1 unit.

$$\underset{(U/L)}{\text{GS activity}} = (\Delta A_{570} - b) \div a \div T \times 1000 \times f$$

2. Tissue sample:

Definition: The amount of GS enzyme in 1 g tissue protein per 1 min that product 1 μmol substrate at 37°C is defined as 1 unit.

$$\underset{(U/gprot)}{\text{GS activity}} = (\Delta A_{570} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

[Note]

ΔA_{570}: $\Delta A_{570} = A_{sample} - A_{control}$.

T: Reaction time, min.

1000： 1 mmol/L = 1000 μmol/L.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	10.00	15.00
%CV	2.0	2.9	4.0

Inter-assay Precision

Three mouse serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	10.00	15.00
%CV	5.3	5.8	6.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	5	10	15
Observed Conc. (U/L)	4.8	9.7	15.0
Recovery rate (%)	96	97	100

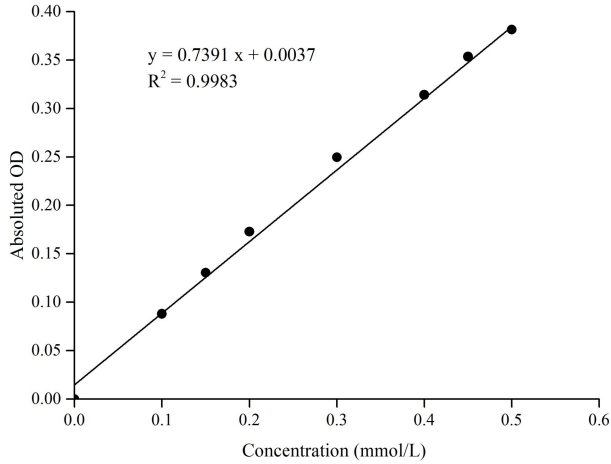
Sensitivity

The analytical sensitivity of the assay is 0.25 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**	**0.45**	**0.5**
OD Value	0.075	0.166	0.208	0.251	0.332	0.385	0.418	0.448
	0.077	0.162	0.205	0.247	0.319	0.395	0.421	0.447
Average OD	0.076	0.164	0.207	0.249	0.326	0.390	0.420	0.448
Absoluted OD	0.000	0.088	0.131	0.173	0.250	0.314	0.344	0.372



Appendix II Example Analysis

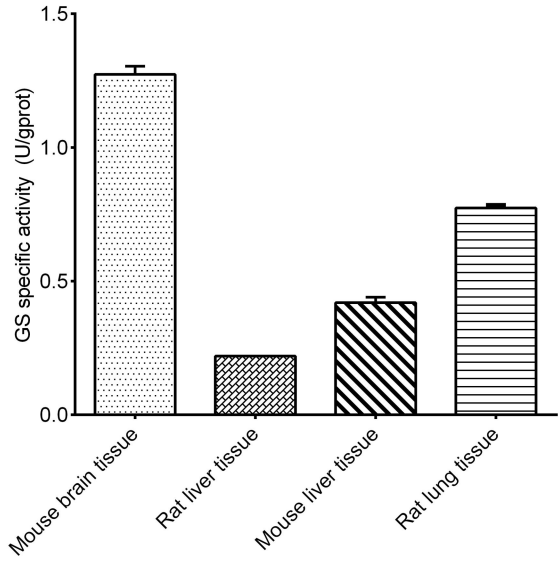
Example analysis：

Take 20 μL of 10% mouse brain tissue homogenate which dilute for 2 times, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.7391 x + 0.0037, The OD value of the sample well is 0.130, the OD value of the control well is 0.056, the concentration of protein is 5.13 gprot/L, and the calculation result is:

$$\text{GS activity (U/gprot)} = (0.130 - 0.056 - 0.0037) \div 0.7391 \div 30 \times 1000 \div 5.13 \times 2 = 1.24 \text{ U/gprot}$$

Detect 10% mouse brain tissue homogenate (the concentration of protein is 5.13 gprot/L, dilute for 2 times), 10% rat liver tissue homogenate (the concentration of protein is 7.74 gprot/L, dilute for 6 times), 10% mouse liver tissue homogenate (the concentration of protein is 11.85 gprot/L, dilute for 6 times) and 10% rat lung tissue homogenate (the concentration of protein is 5.63 gprot/L), according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.