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

Catalog No：E-BC-K660-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.003-18.0 U/L

Elabscience®Glutaminase (GLS) Activity 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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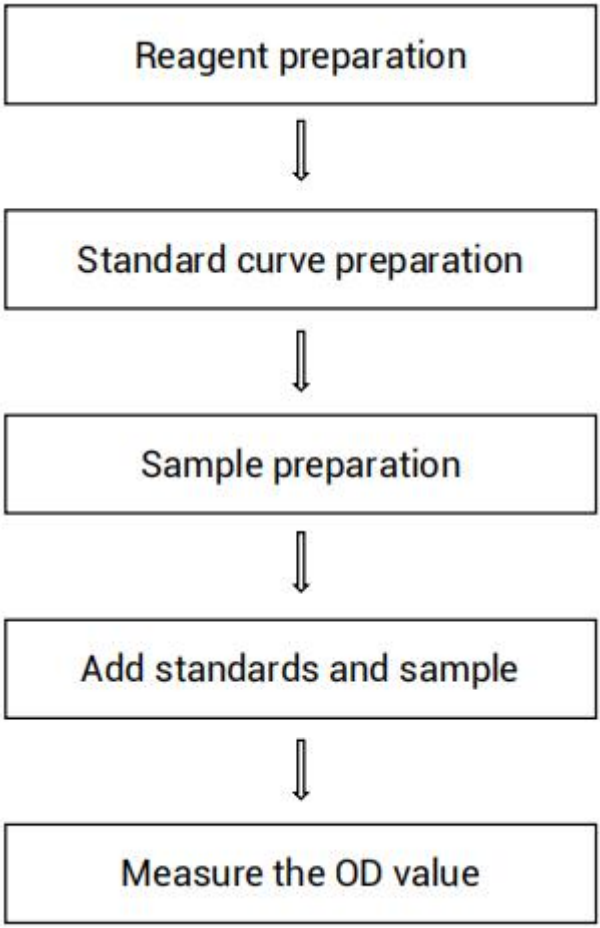
Assay summary



Intended use

This kit can measure glutaminase activity in serum (plasma), animal and plant tissue samples.

Detection principle

Glutamine is decomposed to produce glutamic acid under the action of glutaminase. Glutamic acid is further transformed by glutamic acid dehydrogenase. Meanwhile, NAD^+ is reduced to NADH, Which under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product. The activity of glutaminase can be calculated by measuring the change of absorbance value at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Substrate A	Powder × 2 vials	-20℃, 12 months
Reagent 2	Standard	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Diluent	4 mL×1 vial	-20℃, 12 months
Reagent 4	Enzyme Reagent	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 5	Buffer Solution	20 mL×1 vial	-20℃, 12 months
Reagent 6	Substrate B	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 7	Accelerator	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 8	Chromogenic Agent	1.5 mL × 2 vials	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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:

Incubator, Centrifuge, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

Reagent preparation

① Keep enzyme reagent on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of substrate A working solution：
Dissolve one vial of substrate A with 5 mL of double distilled water, mix well to dissolve. Store at -20°C for 3 days.

③ The preparation of 50 mmol/L standard stock solution：
Dissolve one vial of standard with 1 mL of diluent, mix well to dissolve. Store at 2-8℃ for 3 days.

④ The preparation of 0.5 mmol/L standard solution：
Before testing, please prepare sufficient 0.5 mmol/L standard solution according to the test wells. For example, prepare 1000 μL of 0.5 mmol/L standard solution (mix well 10 μL of 50 mmol/L standard stock solution and 990 μL of double distilled water). The 0.5 mmol/L standard solution should be prepared on spot.

⑤ The preparation of enzyme working solution：

Dissolve one vial of enzyme reagent with 200 μL of double distilled water, mix well to dissolve. The enzyme working solution should be stored at

2-8℃and used up within 6 hours.

⑥ The preparation of substrate B working solution：

Dissolve one vial of substrate B with 400 μL of diluent, mix well to dissolve. Aliquoted storage at -20°C for 3 days protected from light, and avoid repeated freeze/thaw cycles is advised.

⑦ The preparation of accelerator working solution：

Dissolve one vial of accelerator with 1 mL of double distilled water, mix well to dissolve. Store at -20°C for 3 days protected from light.

⑧ The preparation of reaction working solution：

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 747 μL of reaction working solution (mix well 10 μL of enzyme working solution, 690 μL of buffer solution, 37 μL of substrate B working solution and 10 μL of accelerator working solution). Keep reaction working solution on ice during use. The reaction working solution should be prepared on spot and used up within 1 h.

⑨ 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	40	60	80	120	160	180	200
Double distilled water (μL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

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

⑤ 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% Mouse liver tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat kidney tissue homogenate	1
Human serum	1
Human plasma	1-3

Note: The diluent is PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① To avoid contamination, it is recommended to aliquot the accelerator working solution into smaller quantities.

② The incubation process of chromogenic reaction should be with shading light.

Operating steps

Enzymatic reaction

① Sample well: Take 20 μL of sample to the 1.5 mL EP tube.
Control tube: Take 20 μL of sample to the 1.5 mL EP tube.

② Add 80 μL of substrate A working solution to the sample tube, and add 80 μL of double distilled water to the control tube.

③ Mix fully and incubate at 37℃ for 30 min.

④ Centrifuge at 8000 g for 5 min at room temperature. The supernatant is used for chromogenic reaction.

Chromogenic reaction

① Standard well: Take 50 μL of standard solution with different concentrations to the corresponding wells
Sample well: Take 50 μL reaction supernatant of sample tube to the corresponding wells.
Control tube: Take 50 μL reaction supernatant of control tube to the corresponding wells.

② Add 140 μL of reaction working solution to each well.

③ Add 20 μL of chromogenic agent to each well.

④ Mix fully with microplate reader for 3 s and incubate at 37℃ for 20 min with shading light. Measure the OD value of each well at 450 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 (plasma) sample:

Definition: The amount of GLS in 1 L liquid sample per 1 minute that hydrolyze the glutamine to produce 1 μmol glutamic acid at 37°C is defined as 1 unit

$$\text{GLS activity (U/L)} = (\Delta A_{450} - b) \div a \div T \div (V_1 \div V_2) \times f \times 1000*$$

2. Tissue sample:

Definition: The amount of GLS in 1 g tissue protein per 1 minute that hydrolyze the glutamine to produce 1 μmol glutamic acid at 37°C is defined as 1 unit.

$$\text{GLS activity (U/gprot)} = (\Delta A_{450} - b) \div a \div T \div C_{pr} \div (V_1 \div V_2) \times f \times 1000*$$

[Note]

ΔA450: $OD_{Sample} - OD_{Control}$.

T: The time of enzymatic reaction, 30 min

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

V_1: The volume of enzymatic reaction solution added to the chromogenic reaction system, 50 μL.

V_2: Total volume of enzymatic reaction, 100 μL.

f: Dilution factor of sample before test.

1000*: 1 mmol/L =1000 μ mol/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human 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)	0.26	2.80	13.50
%CV	5.4	5.2	4.7

Inter-assay Precision

Three human 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)	0.26	2.80	13.50
%CV	8.2	7.9	8.8

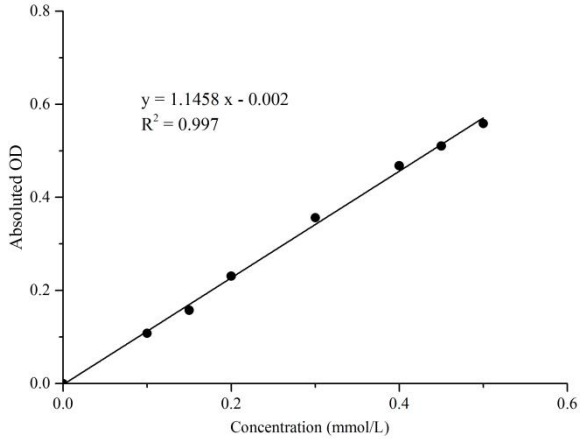
Sensitivity

The analytical sensitivity of the assay is 0.003 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
Average OD	0.067	0.175	0.224	0.298	0.423	0.535	0.577	0.625
Absoluted OD	0.000	0.108	0.158	0.231	0.357	0.468	0.511	0.559



Appendix Π Example Analysis

Example analysis：

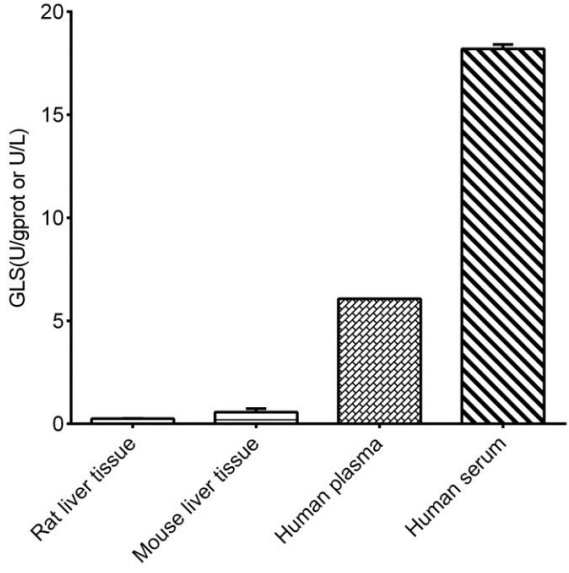
For rat liver tissue, take 20 μL of 10% rat liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 1.1458 x - 0.002, the average OD value of the control is 0.103, the average OD value of the sample is 0.136, the concentration of protein in sample is 6.58 gprot/L, and the calculation result is:

$$\text{GLS activity (U/gprot)} = (0.136 - 0.103 + 0.002) \div 1.1458 \div 30 \div 6.58 \div (50 \div 100) \times 1000$$

$$= 0.309 \text{ U/gprot}$$

Detect 10% rat liver tissue homogenate (the concentration of protein is 6.58 gprot/L), 10% mouse liver tissue homogenate (the concentration of protein is 6.68 gprot/L), human plasma and human serum 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.