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

Catalog No：E-BC-K903-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (440-460 nm)

Detection range：0.006–0.8 mmol/L

Elabscience®Glutamic Acid (Glu) 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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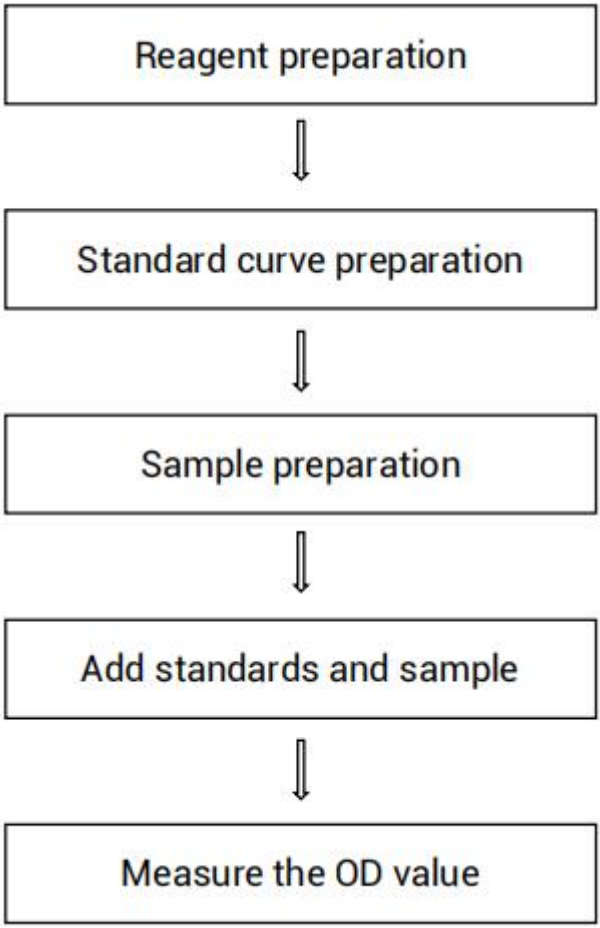
Assay summary



Intended use

This kit can measure glutamic acid (Glu) content in serum (plasma), urine, animal and plant tissue and cells samples.

Detection principle

Glutamic acid can reduce NAD^+ to NADH. NADH, under the action of hydrogen transmitter, reduces WST-8 to form yellow product, which has a characteristic absorption peak at 450 nm. Glutamic acid content can be calculated by measuring the OD value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	8 mL × 1 vial	16 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Substrate	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	1.2 mL × 1 vial	1.2 mL × 2 vials	-20°C, 12 months, shading light
Reagent 5	50 mmol/L Standard	1 mL × 1 vial	1 mL × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	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:

Micropipettor, 37℃ Water bath, Centrifuge, Microplate reader (440-460 nm), Incubator, 10 KD Ultrafiltration tube

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Heat 50 mmol/L Standard in a water bath at 60°C for about 10 minutes until completely dissolved before use. Equilibrate other reagents to room temperature before use.

② Preparation of enzyme working solution：
Dissolve one vial of enzyme reagent with 1.2 mL of double distilled water, mix well to dissolve. Keep enzyme working solution on ice during use Store at -20°C for 7 days protected from light.

③ Preparation of substrate working solution：
Dissolve one vial of substrate with 1.2 mL of double distilled water, mix well to dissolve. Keep substrate working solution on ice during use Store at -20°C for 7 days protected from light.

④ Preparation of reaction working solution：
For each well，prepare 60 uL of reaction working solution(mix well 20 μL of enzyme working solution, 20 μL of substrate working solution and 20 μL of chromogenic agent). The reaction working solution should be prepared on spot. Keep reaction working solution on ice during use and the prepared solution should be used up within 3 hours.

⑤ Preparation of 1 mmol/L standard solution：
Before testing, please prepare sufficient 1 mmol/L standard solution

according to the test wells. For example, prepare 700 μL of 1 mmol/L standard solution(mix well 14 μL of 50 mmol/L standard and 686 μL of double distilled water). The prepared solution should be used up within the same day.

⑥ The preparation of standard curve:

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

Dilute 1 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.8, 0.7, 0.6, 0.5, 0.3, 0.2, 0.1, 0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.5**	**0.6**	**0.7**	**0.8**
1 mmol/L standard (μL)	0	20	40	60	100	120	140	160
Double distilled water (μL)	200	180	160	140	100	80	60	40

Sample preparation

① Sample preparation

Serum, plasma and urine: Filter the sample through a 10 kD ultrafiltration tube and collect the filtrate for detection.

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 normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.
④ Centrifuge at 12000×g for 15 min at 4°C to remove insoluble material. Collect supernatant and filter the supernatant through a 10 kD ultrafiltration tube and collect the filtrate for detection.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
② Wash cells with PBS (0.01 M, pH 7.4).
③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4°C.
④ Centrifuge at 12000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and filter the supernatant through a 10 kD ultrafiltration tube and collect the filtrate for detection.

② **Dilution of sample**

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat brain tissue homogenate	1-2
10% Mouse liver tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat pancreatic tissue homogenate	1
10% Bovine liver tissue homogenate	1
10% Porcine heart tissue homogenate	1
Human serum	3-5
Human urine	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.

Operating steps

① Standard well: Take 30 μL of standard solution with different concentrations into the corresponding wells.
Sample well: Take 30 μL of supernatant of sample into the corresponding wells.

② Add 130 μL of buffer solution to each well.

③ Add 60 μL of reaction working solution into each well.

④ Mix fully for 3 s with microplate reader, incubate at 37℃ for 20 min with shading light and measure the OD values 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:

$$\text{Glu content (mmol/L)} = (\Delta A_{450} - b) \div a \times f$$

2. Tissue sample:

$$\text{Glu content (mmol/kg wet weight)} = (\Delta A_{450} - b) \div a \div \frac{m}{V} \times f$$

3. Cell sample:

$$\text{Glu content (mmol/10^6)} = (\Delta A_{450} - b) \div a \div \frac{n}{V} \times f$$

[Note]

ΔA: $OD_{Sample} - OD_{Blank}$

f: Dilution factor of sample before test

m: The weight of tissue, g

V: The volume of normal saline in the preparation step of sample, mL.

n: The number of cell sample/10^6

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 (mmol/L)	0.04	0.15	0.45
%CV	1.0	0.8	0.6

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 (mmol/L)	0.04	0.15	0.45
%CV	4.3	4.1	4.8

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.4	0.65
Observed Conc. (mmol/L)	0.1	0.4	0.7
Recovery rate (%)	95	99	100

Sensitivity

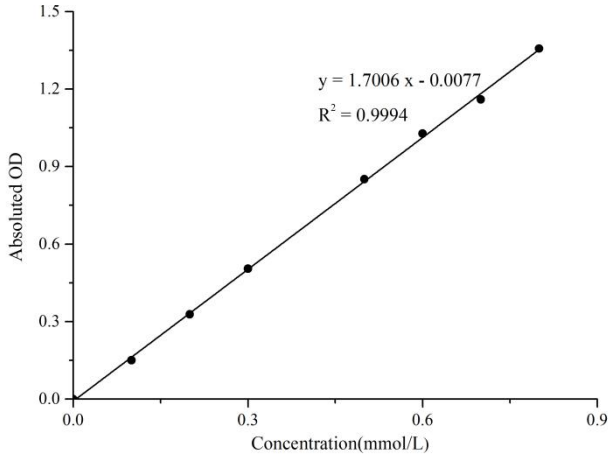
The analytical sensitivity of the assay is 0.006 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

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**	**0.2**	**0.3**	**0.5**	**0.6**	**0.7**	**0.8**
Average OD	0.053	0.203	0.381	0.558	0.904	1.081	1.213	1.410
Absoluted OD	0.000	0.150	0.329	0.505	0.852	1.028	1.160	1.357



Appendix Π Example Analysis

Example analysis：

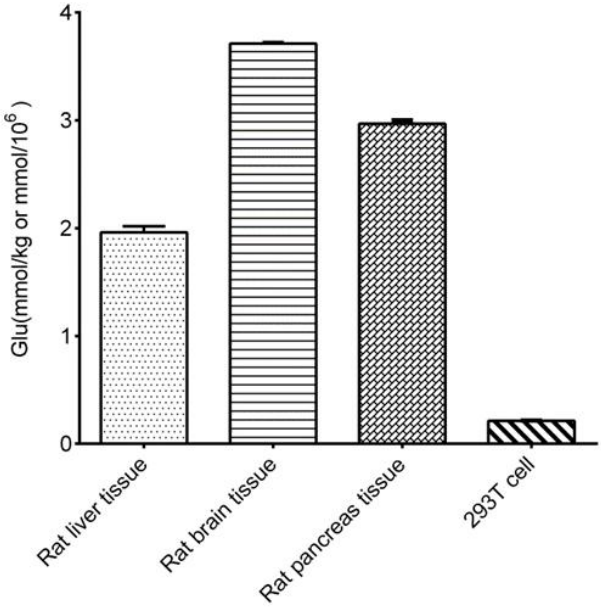
For rat liver tissue, take 30 μL of 10% rat liver tissue homogenate and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 1.7006 x + 0.0077, the average OD value of the blank is 0.053, the average OD value of the sample is 0.416, and the calculation result is:

Glu content (mmol/kg wet weight)

= (0.416 – 0.053 – 0.0077) ÷ 1.7006 ÷ (0.1 ÷ 0.9) = 1.88 mmol/kg wet weight

Detect 10% rat liver tissue homogenate, 10% rat brain tissue homogenate, 10% rat pancreatic tissue homogenate and 293T cell 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.