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

Catalog No：E-BC-K118-S

Specification：50Assays(46 samples)/100Assays(96 samples)

Measuring instrument：Spectrophotometer（340 nm）

Detection range: 4.00-450 μmol/L

Elabscience® Glutamic Acid 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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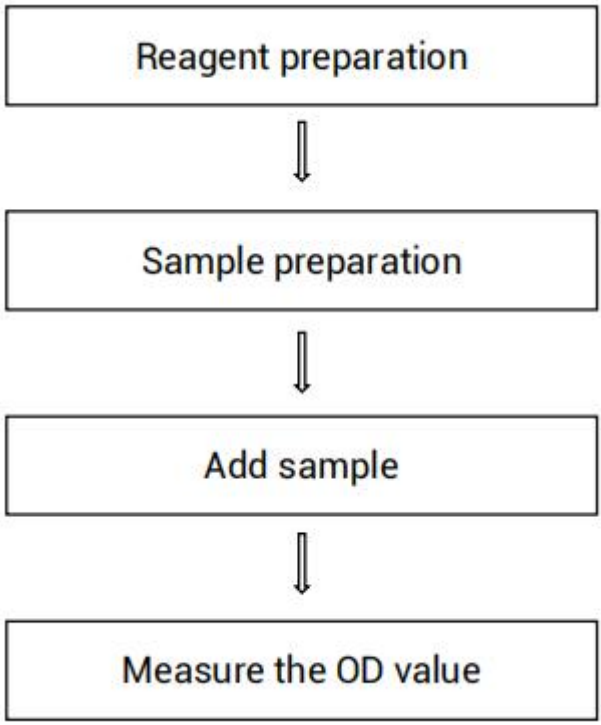
Assay summary



Intended use

This kit can measure glutamic acid content in serum, plasma, milk, animal tissue, cells and cell culture supernatant samples.

Detection principle

Glutamic acid can react with NAD+ under the catalysis of glutamate dehydrogenase to produce α-ketoglutaric acid, NADH and NH4+. NADH has the maximum absorption at 340 nm. And glutamic acid content can be calculated by measuring the change of NADH.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Protein Precipitator	35 mL ×1 vial	35 mL × 2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	60 mL × 1 vial	60 mL × 2 vials	-20℃, 12 months, shading light
Reagent 3	Chromogenic Agent	Powder × 2 vials	Powder × 2 vials	-20℃, 12 months
Reagent 4	Chromogenic Agent Diluent	8 mL × 1 vial	16 mL × 1 vial	-20℃, 12 months
Reagent 5	Accelerator	Powder × 2 vials	Powder × 2 vials	-20℃, 12 months
Reagent 6	Enzyme Reagent	Powder × 2 vials	Powder × 2 vials	-20℃, 12 months
Reagent 7	Enzyme Diluent	1.5 mL × 1 vial	1.5 mL × 2 vials	-20℃, 12 months
Reagent 8	Standard	Powder × 2 vials	Powder × 2 vials	-20℃, 12 months
Reagent 9	Standard Diluent	35 mL × 1 vial	35 mL × 1 vial	-20℃, 12 months

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:

Spectrophotometer (340 nm), Micropipettor, Centrifuge, Incubator, Water bath, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

1. For 50 Assays

① Equilibrate all the reagents to room temperature before use.

② The preparation of chromogenic working solution:
Dissolve one vial of chromogenic with 3 mL of chromogenic agent diluent, mix well. Store at -20°C for 7 days.

③ The preparation of accelerator working solution:
Dissolve one vial of accelerator with 0.3 mL of double distilled water, mix well. Store at -20°C for 7 days.

④ The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 0.6 mL of enzyme diluent, mix well. Keep it on ice during use and store at -20°C for 7 days.

⑤ The preparation of 10 mmol/L standard stock solution:
Dissolve one vial of standard with standard diluent to a final volume of 10 mL in 70-80℃ water bath. Store at 2-8°C for 7 days.

⑥ The preparation of 200 μmol/L standard application solution:
Dilute 0.1mL of 10 mmol/L standard stock solution and 4.9mL of standard diluent, mix well. Store at 2-8°C for 7 days.

⑦ The preparation of reaction working solution:

For each well, prepare 1500 μL of reaction working solution (mix well 1000 μL of buffer solution, 100 μL of chromogenic agent working solution, 10 μL of accelerator working solution and 390 μL of double distilled water).The reaction working solution should be prepared on spot.

2. For 100 Assays

① Equilibrate all the reagents to room temperature before use.

② The preparation of chromogenic working solution:
Dissolve one vial of chromogenic agent with 6 mL of chromogenic agent diluent, mix well to dissolve. Store at -20°C for 7 days.

③ The preparation of accelerator working solution:
Dissolve one vial of accelerator with 0.6 mL of double distilled water, mix well to dissolve. Store at -20°C for 7 days.

④ The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 1.2 mL of enzyme diluent, mix well. Keep it on ice during use and store at -20°C for 7 days.

⑤ The preparation of 10 mmol/L standard stock solution:
Dissolve one vial of standard with standard diluent to a final volume of 10 mL in 70-80℃ water bath. Store at 2-8°C for 7 days.

⑥ The preparation of 200 μmol/L standard application solution:
Add 0.1mL of 10 mmol/L standard stock solution and 4.9mL of standard diluent, mix well. Store at 2-8°C for 7 days.

⑦ The preparation of reaction working solution:
For each well, prepare 1500 μL of reaction working solution (mix well 1000 μL of buffer solution, 100 μL of chromogenic agent working solution, 10 μL of accelerator working solution and 390 μL of double distilled water).The reaction working solution should be prepared on spot.

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°C 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°C.

④ Centrifuge at 10000×g for 10 min 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).

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 300-500 μL (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min 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
Human serum	1
Mouse serum	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat brain tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or 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

The enzyme working solution should be stored in an ice box when perform the experiment.

Operating steps

1. The pretreatment of sample

① For serum (plasma) and cell culture supernatant samples
Take 0.2 mL of sample to 2 mL EP tube, add 0.6 mL of protein precipitator and mix fully, centrifuge at 3100 g for 10 min, then take 0.5 mL of the supernatant for detection.

② For tissue and cell samples
Take 0.2 mL of tissue or cell homogenate sample to 2 mL EP tube, add 0.6 mL of protein precipitator and mix fully, centrifuge at 3100 g for 10 min, then take 0.5 mL of the supernatant for detection.

2. The measurement of sample

① Blank tube: Take 0.5 mL of double distilled water to the tube.
Standard tube: Take 0.5 mL of 200 μmol/L standard application solution to the tube.
Sample tube: Take 0.5 mL of pretreatd sample to the tube.

② Add 1.5 mL of reaction working solution to each tube and vortex with vortex mixer for 3 s.

③ Set the spectrophotometer to zero with double distilled water and measure the OD values (A_1) of each tube at 340 nm wavelength with 1 cm optical path cuvette.

④ Add 0.02 mL of enzyme reagent working solution into each well and vortex with vortex mixer for 3 s.

⑤ Incubate at 37℃ for 40 min.

⑥ Set the spectrophotometer to zero with double distilled water and measure the OD values (A_2) of each tube at 340 nm wavelength with 1 cm optical path cuvette.

Calculation

The sample:

1. Serum (plasma) and other liquid samples

$$\text{Glutamic acid content (μmol/L)} = \frac{(A_{2Sample}-A_{1Sample}) - (A_{2Blank}-A_{1Blank})}{(A_{2Standard}-A_{1Standard}) - (A_{2Blank}-A_{1Blank})} \times c \times 4^{*} \times f$$

2. Tissue and cell samples

$$\text{Glutamic acid content (μmol/gprot)} = \frac{(A_{2Sample} - A_{1Sample}) - (A_{2Blank} - A_{1Blank})}{(A_{2Standard} - A_{1Standard}) - (A_{2Blank} - A_{1Blank})} \times c \times 4^{*} \times f \div C_{pr}$$

[Note]

c: Concentration of standard, 200 μmol/L.

4*: Dilution factor in the step of pretreatment of sample.

f: Dilution factor of sample before test.

C_{pr}: Concentration of the protein in sample, gprot/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 (μmol/L)	25.40	106.80	286.50
%CV	2.5	2.1	2.0

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 (μmol/L)	25.40	106.80	286.50
%CV	3.0	3.1	2.6

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 104%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	84.5	252	347
Observed Conc. (μmol/L)	88.7	257.0	364.4
Recovery rate (%)	105	102	105

Sensitivity

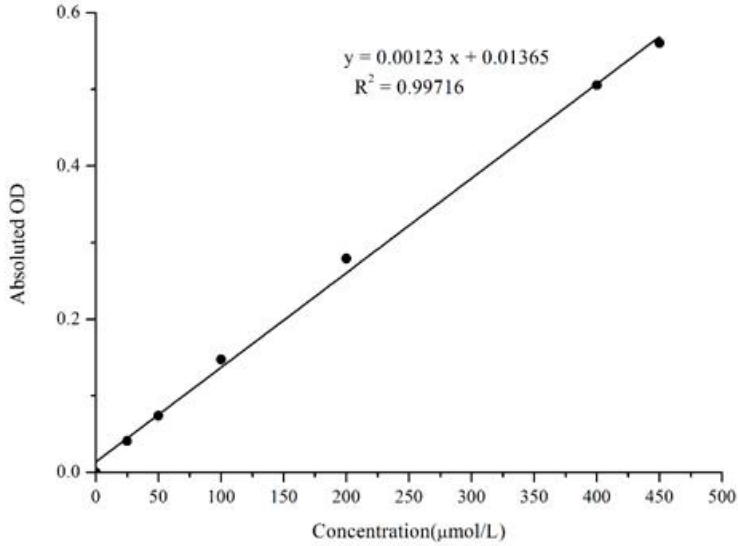
The analytical sensitivity of the assay is 4.00 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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 (μmol/L)	0	25	50	100	200	400	450
Average (A_2-A_1)	0.016	0.057	0.090	0.163	0.295	0.521	0.576
Absoluted OD	0	0.041	0.074	0.147	0.279	0.505	0.560



Appendix Π Example Analysis

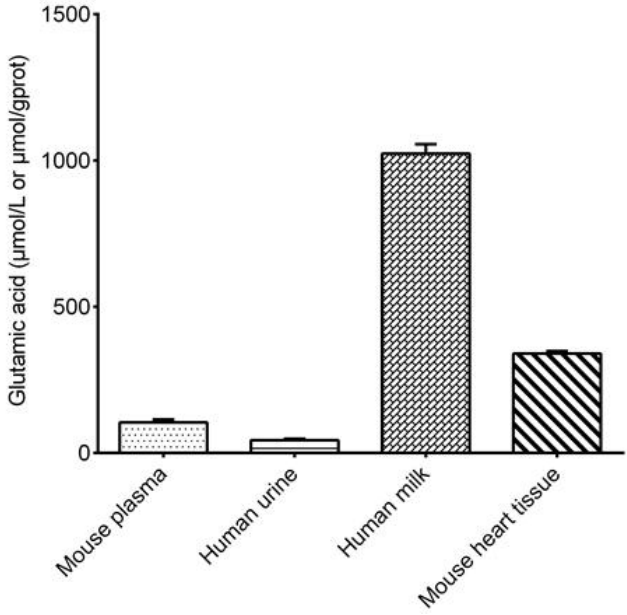
Example analysis：

Take 0.2 mL of mouse plasma and carry the assay according to the operation steps. The results are as follows:

The average A_1 of the sample is 0.047, the average A_2 of the sample is 0.105, the average A_1 of the blank is 0.038, the average A_2 of the blank is 0.060, the average A_1 of the standard is 0.070, the average A_2 of the standard is 0.339, and the calculation result is:

$$\text{Glutamic acid content (μmol/L)} = \frac{(0.105 - 0.047) - (0.060 - 0.038)}{(0.339 - 0.070) - (0.060 - 0.038)} \times 200 \times 4 = 116.60 \text{ μmol/L}$$

Detect mouse plasma, human urine, human milk and 10% mouse heart tissue homogenate (the concentration of protein in sample is 3.76 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.