PRODUCT INFORMATION & MANUAL

Glutamic Acid Assay Kit (Colorimetric)

NBP3-25810

For research use only.
Not for diagnostic or therapeutic procedures.

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Glutamic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25810

Method: Colorimetric method

Specification: 100Assays (Can detect 96 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 4.00 μmol/L

Detection range: 4.00-450 μmol/L

Average intra-assay CV (%): 2.2

Average inter-assay CV (%): 2.9

Average recovery rate (%): 104

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

Glutamate is a dicarboxylic acid, the most abundant amino acid in the cell, which can be converted into aminobutyric acid (GABA), ornithine, ketoglutarate, glucose or glutathione. Glutamate links carbohydrate and amino acid metabolism through the tricarboxylic acid (TCA) cycle. In the liver, it can regulate the rate of ammonia to urea. In the central nervous system, it can act as an excitatory neurotransmitter.

▲Detection principle

Glutamic acid can react with NAD^+ under the catalysis of glutamate dehydrogenase to produce α-ketoglutaric acid, NADH and NH_4^+. 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	Specification	Storage
Reagent 1	Protein Precipitator	35 mL × 2 vials	-20℃ , 12 months
Reagent 2	Buffer Solution	60 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 3	Chromogenic Agent	Powder × 2 vials	-20℃ , 12 months
Reagent 4	Chromogenic Agent Diluent	16 mL × 1 vial	-20℃ , 12 months
Reagent 5	Accelerator	Powder × 2 vials	-20℃ , 12 months
Reagent 6	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months
Reagent 7	Enzyme Diluent	1.5 mL × 2 vials	-20℃ , 12 months
Reagent 8	Standard	Powder × 2 vials	-20℃ , 12 months
Reagent 9	Standard Diluent	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.

▲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)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key point of the assay

The reagent 6 working solution should be stored in an ice box when perform the experiment.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 3 working solution:

Dissolve a vial of reagent 3 powder with 6 mL of reagent 4 and mix fully. The prepared reagent 3 working solution can be store at -20℃ for 7 days.

2. Preparation of reagent 5 working solution:

Dissolve a vial of reagent 5 powder with 0.6 mL of double distilled water and mix fully. The prepared reagent 5 working solution can be store at -20℃ for 7 days.

3. Preparation of reagent 6 working solution:

Dissolve a vial of reagent 6 powder with 1.2 mL of reagent 7 and mix fully. The prepared reagent 6 working solution should be store at -20℃ for 7 days.

4. Preparation of 10 mmol/L standard stock solution:

Dissolve a vial of reagent 8 powder with reagent 9 to a final volume of 10 mL in 70-80℃ water bath. The prepared solution can be stored at 2-8℃ for 7 days.

5. Preparation of 200 μmol/L standard application solution:

Dilute 10 mmol/L standard stock solution with reagent 9 for 50 times. The prepared 200 μmol/L standard application solution can be stored at 2-8℃ for 7 days.

6. Preparation of reaction working solution:

Mix reagent 2, reagent 3 working solution, reagent 5 working solution, double distilled water at the ratio of reagent 2: reagent 3 working solution: reagent 5 working solution: double distilled water = 1: 0.1: 0.01: 0.39. Prepare the fresh needed amount solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (4.00-450 μmol/L).

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).

Assay protocol

▲Detailed operation 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 reagent 1 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 reagent 1 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 reagent 6 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.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.5		
200 μmol/L standard application solution (mL)		0.5	
Pretreatd sample (mL)			0.5
Reaction working solution (mL)	1.5	1.5	1.5
Vortex. Set the spectrophotometer to zero and measure the OD values (A_1) of each tube.			
Reagent 6 working solution (mL)	0.02	0.02	0.02
Vortex, incubate at 37℃ for 40 min. Set the spectrophotometer to zero and measure the OD values (A_2) of each tube.			

▲Calculation

1. For serum (plasma) and other liquid samples

$$\text{Glutamic acid content (μmol/L)} = \frac{(A2_{Sample} - A1_{Sample}) - (A2_{Blank} - A1_{Blank})}{(A2_{Standard} - A1_{Standard}) - (A2_{Blank} - A1_{Blank})} \times c \times 4^{*} \times f$$

2. For tissue and cell samples

$$\text{Glutamic acid content (μmol/gprot)} = \frac{(A2_{Sample} - A1_{Sample}) - (A2_{Blank} - A1_{Blank})}{(A2_{Standard} - A1_{Standard}) - (A2_{Blank} - A1_{Blank})} \times c \times 4^{*} \times f \div C_{pr}$$

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.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.

Appendix I Data

▲Example analysis

Take 0.2 mL of mouse plasma and carry the assay according to the operation table. 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:

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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Milk

Collect fresh milk, centrifuge at 10000 g for 10 min at 4℃ , remove the upper layer of milky white, take the middle layer supernatant and preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲ Cell culture supernatant

Detect the cell culture supernatant directly. If there is turbidity, centrifuge at 3100 g for 10 min, take the supernatant and preserve it on ice for detection. If not detected on the same day, stored the serum at -80℃ , which can be stored for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲ Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Newsholme P, Lima M M R, Procopio J, et al. Glutamine and glutamate as vital metabolites[J]. Brazilian Journal of Medical & Biological Research, 2003, 36(2): 153-163.
2. Newsholme P, Procopio J, Lima M M, et al. Glutamine and glutamate--their central role in cell metabolism and function[J]. Cell Biochemistry & Function, 2010, 21(1): 1-9.
3. Schousboe A, Scafidi S, Bak L K, et al. Glutamate Metabolism in the Brain Focusing on Astrocytes[J]. Advances in Neurobiology, 2014, 11: 13-30.
4. Meldrum B S. Glutamate as a neurotransmitter in the brain: review of physiology and pathology[J]. Journal of Nutrition, 2000, 130(4S Suppl): 1007S-1015S.