PRODUCT INFORMATION & MANUAL

Glutamic Acid Assay Kit (Colorimetric)

NBP3-25805

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

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Glutamic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25805

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.006 mmol/L

Detection range: 0.006–0.8 mmol/L

Average intra-assay CV (%): 0.8

Average inter-assay CV (%): 4.4

Average recovery rate (%): 98

▲ 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 (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	Specification	Storage
Reagent 1	Buffer Solution	16 mL × 1 vial	-20℃ , 12 months
Reagent 2	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 3	Substrate	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Chromogenic Agent	1.2 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 5	50 mmol/L Standard	1 mL × 2 vials	-20℃ , 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.			

▲Materials prepared by users

Instruments

Micropipettor, 37℃ Water bath, Centrifuge, Microplate reader (440-460 nm),

⚠ 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.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use. Heat reagent 5 in a water bath at 60°C for about 10 minutes until completely dissolved before use.
2. Preparation of reagent 2 working solution:
   Dissolve a vial of reagent 2 powder with 1.2 mL of double distilled water and mix fully. Preserve it on ice for use. The prepared reagent 2 working solution can be stored at -20°C for 7 days with shading light.
3. Preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 powder with 1.2 mL of double distilled water and mix fully. Preserve it on ice for use. The prepared reagent 3 working solution can be stored at -20°C for 7 days with shading light.
4. Preparation of reaction working solution:
   Mix reagent 2 working solution, reagent 3 working solution, reagent 4 at the ratio of 1: 1: 1. Prepare the fresh needed amount solution before use. Preserve it on ice for use and it should be used up within 3 h.
5. Preparation of 1 mmol/L standard solution:
   Dilute 50 mmol/L standard with double distilled water for 50 times. Prepare the fresh needed amount solution before use and the prepared solution should be used up on the same day.

▲ Sample preparation

1. Serum, plasma and other liquid:

Filter the sample through a 10 kD ultrafiltration tube and collect the filtrate for detection.

2. 10% tissue homogenate sample:

Accurately weigh the tissue sample, add 9 times the volume of normal saline (0.9%NaCl) according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 12000 g for 15 min, then take the supernatant. Filter the supernatant through a 10 kD ultrafiltration tube and collect the filtrate for detection.

3. Cell sample:

Wash the cells with PBS (0.01 M, pH7~7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add normal saline (0.9%NaCl) at a ratio of cell number (10^6): normal saline (0.9%NaCl) (μL) =1: 200. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 12000 g for 15 min, then take the supernatant. Filter the supernatant through a 10 kD ultrafiltration tube and collect the filtrate for detection.

▲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 (0.006–0.8 mmol/L).

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).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

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:

Number	Standard concentrations (mmol/L)	1 mmol/L Standard (μL)	Double distilled water (μL)
A	0	0	200
B	0.1	20	180
C	0.2	40	160
D	0.3	60	140
E	0.5	100	100
F	0.6	120	80
G	0.7	140	60
H	0.8	160	40

2. The measurement of samples

(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 reagent 1 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°C for 20 min with shading light and measure the OD values of each well at 450 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Standards solution with different concentrations (μL)	30	
Sample (μL)		30
Reagent 1 (μL)	130	130
Reaction working solution (μL)	60	60
Mix fully, incubate at 37°C for 20 min with shading light and measure the OD values of each well.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. For serum/plasma samples :

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

2. For tissue sample:

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

3. For cell sample:

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

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA: OD_{Sample} – OD_{Blank}.

f: Dilution factor of sample before test.

m: The weight of tissue sample, g.

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

n: The number of cell sample/10^6.

Appendix I Data

▲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