Technical Manual

Glutamic Acid Colorimetric Assay Kit

- **Catalogue Code: MAES0098**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

6.43-407 μmol/L

Sensitivity:

6.43 μmol/L

Storage:

-20°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. Detection Principle

Glutamic acid can react with NAD^+ 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.

5. Kit components & storage

Item	Specification	Storage
Protein Precipitator	40 mL × 1 vial	-20°C, 3 months
Buffer Solution	12 mL ×1 vial	-20°C, 3 months, shading light
Chromogenic Agent	Powder × 2 vials	-20°C, 3 months
Chromogenic Agent Diluent	1.5 mL × 2 vials	-20°C, 3 months
Accelerator	Powder × 2 vials	-20°C, 3 months
Enzyme Reagent	Powder × 2 vials	-20°C, 3 months
Enzyme Diluent	1.0 mL × 2 vials	-20°C, 3 months
Standard	Powder × 2 vials	-20°C, 3 months
Standard Diluent	20 mL × 1 vial	-20°C, 3 months
UV Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (330-350 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

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

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of chromogenic agent working solution: Dissolve a vial of chromogenic agent powder with 1.2 mL of chromogenic agent diluent and mix fully. The prepared chromogenic agent working solution can be stored at -20°C for 7 days.

3. Preparation of accelerator working solution: Dissolve a vial of accelerator powder with 0.2 mL of double distilled water and mix fully. The prepared accelerator working solution can be stored at -20°C for 7 days.

4. Preparation of enzyme working solution: Dissolve a vial of enzyme reagent powder with 0.6 mL of enzyme diluent and mix fully, preserve it on ice for detection. The prepared enzyme working solution can be stored at -20°C for 7 days.

5. Preparation of 10 mmol/L standard stock solution: Dissolve a vial of standard powder with 5 mL of standard diluent in 70-80°C water bath. After cool for detection and the prepared solution can be stored at 2-8°C for 7 days.

6. Preparation of 1 mmol/L standard solution: Dilute 10 mmol/L standard stock solution with standard diluent for 10 times. The prepared solution can be stored at 2-8°C for 7 days.

7. Preparation of reaction working solution: Mix buffer solution, chromogenic agent working solution, accelerator working solution, double distilled water at the ratio of 9: 2: 0.1: 3.9. Prepare the fresh needed amount solution before use.

8. Sample Preparation

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. 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°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4°C. 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°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8°C) (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 (E-BC-K318-M).

4. 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 PBS at a ratio of cell number (10^6): PBS (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 (E-BC-K318-M).

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (6.43-407 μmol/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 brain tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse kidney tissue homogenate	1
Jurkart cell supernatant	1-5
Rat serum	1-5
Rat plasma	1
HL-60 cell supernatant	5-15
10% Rat heart tissue homogenate	1
10% Rat stomach tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 340 nm

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.

10. Operation Steps

The pre-treatment of sample

1. For serum (plasma) and cell culture supernatant samples:
Take 0.05 mL of sample to 2 mL EP tube, add 0.15 mL of protein precipitatorr and mix fully, centrifuge at 3100 g for 10 min, then take 0.05 mL of the supernatant for detection.

2. For tissue and cell samples:
Take 0.05 mL of tissue or cell homogenate sample to 2 mL EP tube, add 0.15 mL of protein precipitatorr and mix fully, centrifuge at 3100 g for 10 min, then take 0.05 mL of the supernatant for detection.

The preparation of standard curve

Dilute standard solution (1 mmol/L) with standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 25, 50, 100, 200, 300, 400, 450 μmol/L.

The measurement of samples

1. **Standard well:** Add 50 μL of standard solution with different concentrations to the corresponding wells.
   Sample well: Add 50 μL of sample supernatant to the corresponding wells.

2. Add 150 μL of reaction working solution to each well.

3. Mix fully for 5s with microplate reader and measure the OD values of each well at 340 nm with microplate reader, recorded as A_1.

4. Add 10 μL of enzyme working solution into each well.

5. Mix fully for 5s with microplate reader and incubate at 37°C for 40 min. Measure the OD values of each well at 340 nm with microplate reader, recorded as A_2. $\Delta A = A_2 - A_1$.

Operation Table

	Standard well	Sample well
Standard solution with different concentrations (μL)	50	
Sample supernatant (μL)		50
Reaction working solution (μL)	150	150
Mix fully for 5s with microplate reader and measure the OD values of each well at 340 nm with microplate reader, recorded as A_1.		
Enzyme working solution (μL)	60	60
Mix fully for 5s with microplate reader and incubate at 37°C for 40 min. Measure the OD values of each well at 340 nm with microplate reader, recorded as A_2. $\Delta A = A_2 - A_1$.		

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11. Calculations

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. Serum (plasma) and other liquid sample:

Glutamic acid content (μmol/L)
= (ΔA ' - b) ÷ a ×4 × f

2. Tissue and cell sample:

Glutamic acid content (μmol/gprot)
= (ΔA ' - b) ÷ a ×4 × f ÷ C_{pr}

y: $\Delta A_{Standard}$ - ΔA_{Blank} (ΔA_{Blank} is the change of 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 ' : ΔA ' =ΔA_{Sample} - ΔA_{Blank}

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

f: Dilution factor of sample before test

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

12. Performance Characteristics

Detection Range	6.43-407 μmol/L
Sensitivity	6.43 μmol/L
Average recovery rate (%)	98
Average inter-assay CV (%)	8.3
Average intra-assay CV (%)	2.5

Analysis

Take 50 μL of 10% rat liver tissue homogenate supernatant, carry out the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.0007 x + 0.0046, the change OD value of the sample is 0.138, the change OD value of the blank is 0.018, the concentration of protein in sample is 8.52 gprot/L, and the calculation result is:

Glutamic acid content (μmol/gprot)
= （0.138-0.018-0.0046）÷ 0.0007 × 4 ÷ 8.52
= 77.39 μmol/gprot

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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