TECHNICAL MANUAL

Glutamic Acid (Glu) Colorimetric Assay Kit

- **SKU CODE:** MAES0249
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure the absorbance at 450 nm

2. Intended use

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

3. Detection principle

Glutamic acid can reduce NAD+ to NADH. NADH, under the action of hydrogen transmitter, reduces WST-8 to form a 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.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	8 mL x 1 vial	16 mL x 1 vial	-20°C, 12 months, shading light
Reagent 2	Enzyme Reagent	Powder x 1 vial	Powder x 2 vials	-20°C, 12 months, shading light
Reagent 3	Substrate	Powder x 1 vial	Powder x 2 vials	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	1.2 mL x 1 vial	1.2 mL x 2 vials	-20°C, 12 months, shading light
Reagent 5	50 mmol/L Standard	1 mL x 1 vial	1 mL x 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Micropipettor, 37°C water bath, centrifuge, microplate reader (440-460 nm), incubator, 10 kD ultrafiltration tube

Reagents:

Double distilled water, normal saline (0.9% NaCl)

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 small volume reagents, please centrifuge before use to ensure sufficient amount of reagents.

6. 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 µL 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.

7. Standard curve preparation

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

8. Sample preparation

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 an 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.

Dilution of sample:

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

- 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.

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

10. 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:

Glu content (mmol/L) = (ΔA_450 - b) ÷ a × f

2. Tissue sample:

Glu content (mmol/kg wet weight) = (ΔA_450 - b) ÷ a ÷ (m/V) × f

3. Cell sample:

Glu content (mmol/10^6) = (ΔA_450 - b) ÷ a ÷ (n/V) × 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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision Results

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.

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.04	0.15	0.45
%CV	4.3	4.1	4.8

Recovery

Three samples of high concentration, middle concentration and low concentration were tested in 6 parallel replicates for each concentration to obtain an average recovery rate of 98%.

Recovery Results

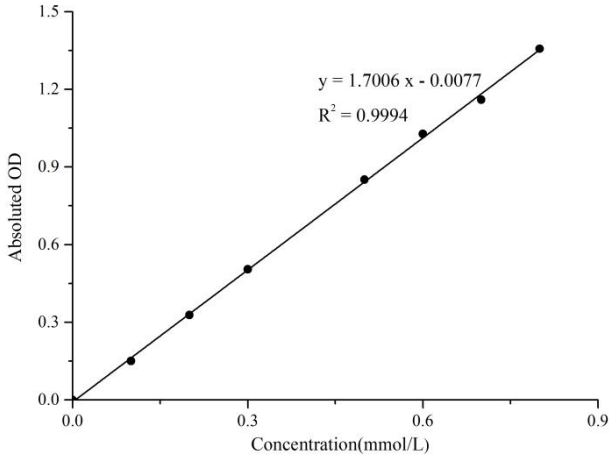
Standard	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Standard 1	0.15	0.1	95
Standard 2	0.4	0.4	99
Standard 3	0.65	0.7	100

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.

Standard curve data

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), the standard curve and data are provided below for reference only:



Standard curve reference data

Concentration (mmol/L)	Average OD	Absoluted OD
0	0.053	0.000
0.1	0.203	0.150
0.2	0.381	0.329
0.3	0.558	0.505
0.5	0.904	0.852
0.6	1.081	1.028
0.7	1.213	1.160
0.8	1.410	1.357

12. Appendix II Example Analysis

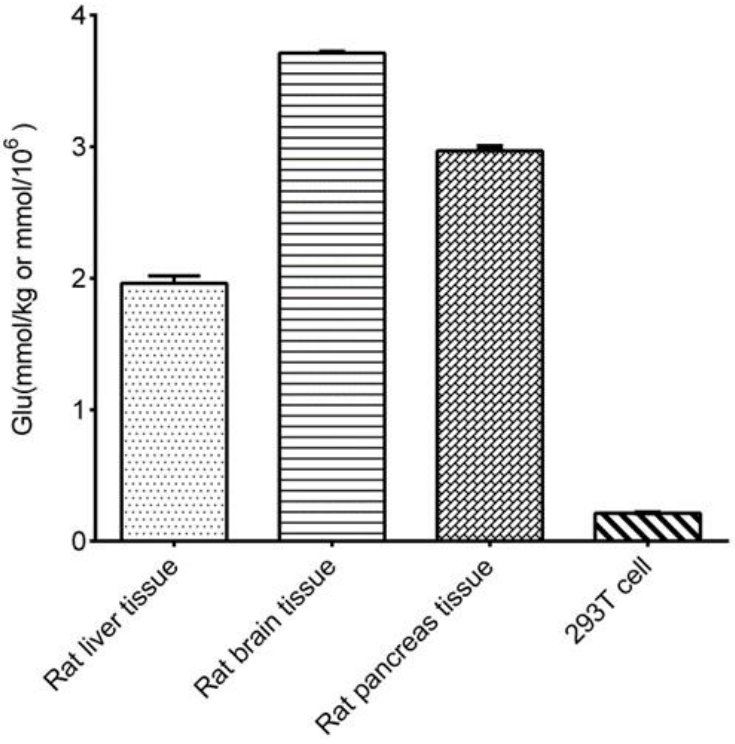
Example analysis:

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

Standard curve: y = 1.7006x + 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:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.