TECHNICAL MANUAL

gamma-Glutamyl Transferase (gamma-GT) Activity Assay Kit

- **SKU CODE:** MAES0103
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add samples and standards
- Incubate and measure absorbance
- Calculate GGT activity

2. Intended use

This kit can measure γ-glutamyl transferase (γ-GT) activity in serum, plasma, and animal tissue samples.

3. Detection principle

γ-GT catalyzes the transfer of the gamma glutamyl group from glutamyl p-nitroaniline to N-glycyl glycine to produce p-nitroaniline, which has a characteristic absorption peak at 405 nm. The activity of γ-GT can be calculated according to the changing rate of absorbance value.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	2-8°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, protect from light
Reagent 3	Extracting Solution	50 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 4	1.0 mmol/L p-Nitroaniline Standard Solution	1.5 mL × 1 vial	1.5 mL × 1 vial	2-8°C, 12 months
Reagent 5	Standard Diluent	10 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (405 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water

6. Reagent preparation

1. **Equilibrate all reagents to room temperature before use.:** Preheat the 1.0 mmol/L p-nitroaniline standard solution and standard diluent at 37°C until clarified before use.
2. **Preparation of substrate application solution::** Dissolve one vial of substrate with 3 mL of standard diluent, mix well. Store aliquots at 2-8°C for 7 days, and avoid repeated freeze/thaw cycles.
3. **Preparation of reaction working solution::** For each well, prepare 250 µL of reaction working solution (mix well 200 µL of buffer solution and 50 µL of substrate application solution). The reaction working solution should be prepared immediately before use.
4. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1.0 mmol/L p-nitroaniline standard solution with standard diluent to serial concentrations. The recommended dilution gradient is as follows: 0, 200, 400, 500, 600, 800, 900, 1000 µmol/L.

7. 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 one 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 extracting solution with a dounce homogenizer at 4°C. 4. Centrifuge at 10,000×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 (MAES0177).
2. **Dilution of sample:** The recommended dilution factor for different samples is 1:1 (no dilution) for most sample types including mouse serum, human serum, rat

serum, dog serum, human plasma, horse serum, porcine serum, human hydrothorax, and various tissue homogenates. The diluent is extracting solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Key points of the assay

1. The temperature and time of incubation at 37°C must be accurate.

2. If the γ-GT activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (MAES0177).

3. Accurate operation is required when adding liquid to the microplate and prevent the formation of bubbles when adding the liquid to the microplate.

4. It is recommended to extend the reaction time of A2 to 15 min for low content samples.

9. Operating steps

1. **Preparation of standard working solution:** For each well, prepare 250 μL of standard working solution (mix well 200 μL of buffer solution and 50 μL of standard solutions with different concentrations).
2. **The measurement of samples:** Standard well: add 25 μL of double distilled water to the corresponding wells. Sample well: add 25 μL of sample to the corresponding wells.
3. **Add working solutions:** Standard well: add 250 μL of standard working solution with different concentrations to standard wells. Sample well: add 250 μL of reaction working solution to sample wells.
4. **Mix and incubate:** Mix fully for 10 s with microplate reader, incubate at 37°C for 1 min.
5. **Measure absorbance:** Measure the OD value (A1) of each well at 405 nm, then incubate the microplate at 37°C for 5 min accurately and measure the OD value (A2) of each well at 405 nm. ΔA=A2-A1. (Note: Standard wells only need to measure the OD values of A2. It is recommended to extend the reaction time of A2 to 15 min for low content samples.)

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 absolute OD value.

3. Plot the standard curve by using absolute 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) and other liquid sample:

Definition: The amount of 1 μmol of p-nitroaniline catalyzed by 1 L of sample per minute is defined as 1 unit.

γ-GT activity (U/L) = (ΔA_sample - b) ÷ a × V_1 ÷ V_2 ÷ T × f

= 0.4 × (ΔA_sample - b) ÷ a × f

2. Tissue sample:

Definition: The amount of 1 μmol of p-nitroaniline catalyzed by 1 g of protein per minute is defined as 1 unit.

γ-GT activity (U/gprot) = (ΔA_sample - b) ÷ a × V_1 ÷ V_2 ÷ C_{pr} ÷ T × f

= 0.4 × (ΔA_sample - b) ÷ a ÷ C_{pr} × f

[Note]

f: Dilution factor of sample before tested.

ΔA_sample: $A_2 – A_1$.

V_1: The volume of substrate application solution, 50 μL = 5.0×10^{-5} L.

(Reaction working solution was mixed buffer solution and substrate application solution at the ratio of 4:1).

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

V_2: The volume of sample added to the reaction, 25 μL = 2.5×10^{-5}L.

T: reaction time, 5 min.

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)

Inter-assay Precision

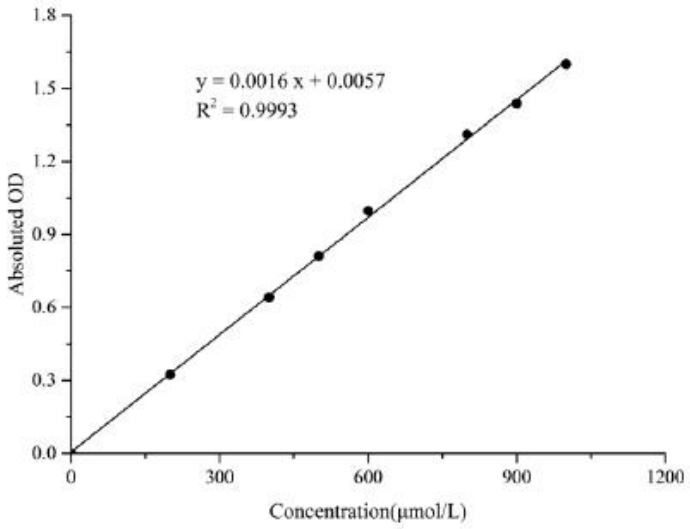
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.50	94.60	207.00
%CV	6.0	6.3	6.3

Sensitivity

The analytical sensitivity of the assay is 0.88 U/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

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:



12. Appendix II Example Analysis

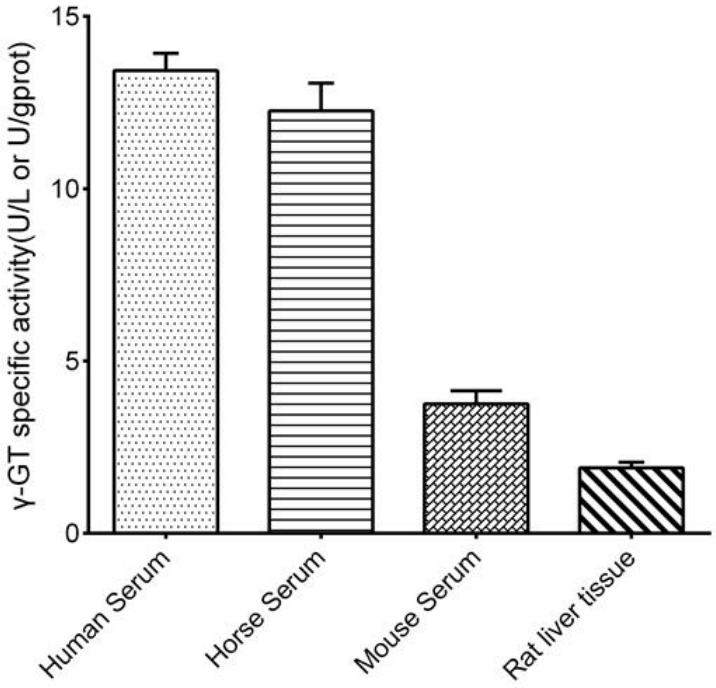
Example analysis:

For human serum, take 25 μL of human serum and carry out the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0016x + 0.0057, the average OD value of the sample incubation for 1 min (A_1) is 1.111, the average OD value incubation for 5 min (A_2) is 1.159, then $\Delta A = A_2 - A_1 = 0.048$, and the calculation result is:

γ-GT activity (U/L) = 0.4 × (0.048 - 0.0057) ÷ 0.0016 = 10.575 U/L

Detect human serum, horse serum, mouse serum and 10% rat liver tissue homogenate (the concentration of protein is 5.36 g/L) 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.