Technical Manual

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

- Catalogue Code: MAES0103
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.88-399.4 U/L

Sensitivity:

0.88 U/L

Storage:

2-8°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.

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2. Background

γ-Glutamyl transferase (γ-GT) is widely present in various organs of the human body. It is a key enzyme in the γ-glutamyl cycle. It catalyzes the degradation of GSH and participates in regulating the level of glutathione in tissues and the absorption of amino acids. And excretion, and the acylation of free amino acids in the peptide chain. The activity of normal human serum γ-GT is very low. In patients with acute hepatitis, liver cancer, and obstructive yellow pox, the serum γ-GT activity is significantly increased. Therefore, the determination of γ-GT activity has certain significance for the diagnosis of hepatobiliary system diseases. In combination with other enzyme activity determination, it is helpful for the diagnosis of liver cancer.

3. Intended Use

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

4. Detection Principle

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

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	30 mL x 1 vial	2-8°C, 3 months
Substrate	Lyophilized × 2 vials	2-8°C, 3 months, avoid direct sunlight
Extracting Solution	50 mL × 2 vials	2-8°C, 3 months
p-Nitroaniline Standard Solution (1.0 mmol/L)	1.5 mL x 1 vial	2-8°C, 3 months
Standard Diluent	10 mL x 1 vial	2-8°C, 3 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (405 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

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 microplate and prevent the formulation of bubbles when adding the liquid to the microplate.

4. It is recommended to extend the reaction time of A_2 to 15min for low content samples

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate solution:** Dissolve a vial of substrate with 3 mL of standard diluent and mix fully. The prepared solution can be stored at 2-8°C for a month.

3. Preparation of **reaction working solution:** Mix the buffer solution and substrate solution at the ratio of 4:1 fully. Prepare the fresh 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, 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:
Weigh the tissue accurately and add extracting solution at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for measurement.

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 (0.88-399.4 U/L).

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

Sample Type:	Dilution Factor
Mouse serum	1
Human serum	1
Rat serum	1
Dog serum	1
Human plasma	1
Horse serum	1
Porcine serum	1
Human hydrothorax	1
10% Mouse liver tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat lung tissue homogenate	1

Note: The diluent is extracting solution;

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 405 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 preparation of standard curve

Dilute p-nitroaniline standard solution (1.0 mmol/L) with standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 200, 400, 500, 600, 800, 900, 1000 μmol/L.

Preparation of standard working solution

Mix the above diluted standard solutions with different concentrations and buffer solution in a ratio of buffer solution: standard solution=4:1, prepare the fresh solution before use.

The measurement of samples

1. **Standard well:** add 25 μL of double distilled water to the corresponding wells.
   Sample well: add 25 μL of sample to the corresponding wells.

2. **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.

3. Mix fully for 10 s with microplate reader, incubate at 37°C for 1 min accurately and measure the OD value (A_1) of each well at 405 nm, then incubate the microplate at 37°C for 5 min accurately and measure the OD value (A_2) of each well at 405 nm. $\triangle A=A_2-A_1$. (Note: Standard wells only need to measure the OD values of A_2).

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Operation Table

	Sample well	Standard well
Double distilled water (μL)	25	
Sample (μL)		25
Standard working solution with different concentrations (μL)	250	
Reaction working solution (μL)		250
Mix fully for 10 s with microplate reader, incubate at 37°C for 1 min accurately and measure the OD value (A_1) of each well at 405 nm, then incubate the microplate at 37°C for 5 min accurately and measure the OD value (A_2) of each well at 405 nm. $\triangle A=A_2-A_1$.		

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:

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)

$=(\triangle A_{sample} - b) \div a \times V_2 \div V_{sample} \div T \times f$

$= 0.4 \times (\triangle A_{sample} - b) \div a \times f$

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)

$=(\triangle A_{sample} - b) \div a \times V_2 \div V_{sample} \div Cpr \div T \times f$

$= 0.4 \times (\triangle A_{sample} - b) \div a \div Cpr \times f$

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.

f: Dilution factor of sample before tested.

ΔA_{sample}: $A_2 - A_1$.

V_2: The volume of substrate solution, 50 μL = 5.0×10^{-5} L. (Reaction working solution was mixed buffer solution and substrate solution at the ratio of 4:1)

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

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

T: reaction time, 5 min.

12. Performance Characteristics

Detection Range	0.88-399.4 U/L
Sensitivity	0.88 U/L
Average inter-assay CV (%)	6.2
Average intra-assay CV (%)	4.2

Analysis

For human serum, take 25 μL of human serum, carry the assay according to the operation table.

The results are as follows:

$y = 0.0016x - 0.0057$, 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 $\triangle A = A_2 - A_1 = 0.048$, and the calculation result is:

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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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Notes:

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Notes:

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