（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K126-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（405 nm）

Detection range：0.88-399.4 U/L

Elabscience® γ-Glutamyl Transferase (GGT/γ-GT) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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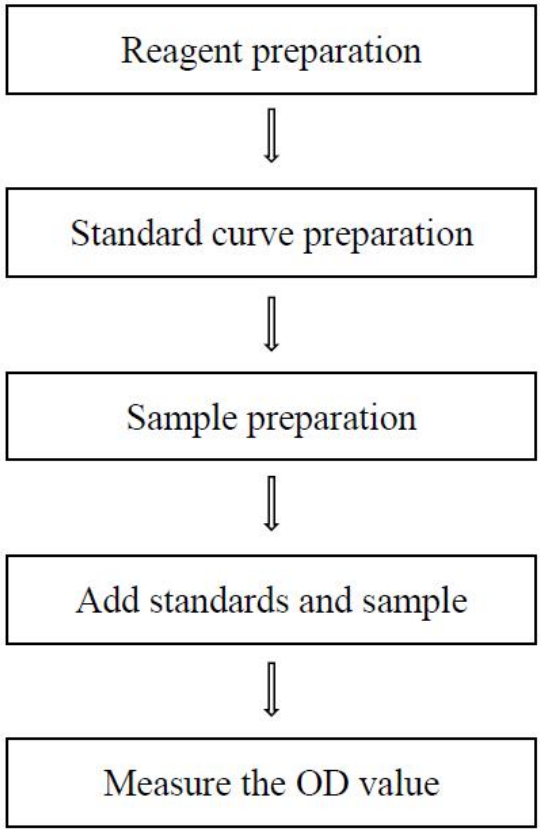
Assay summary



Intended use

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

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.

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, shading 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		

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 a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all the 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 The preparation of substrate application solution：

Dissolve one vial of substrate with 3 mL of standard diluent, mix well. Aliquoted storage at 2-8°C for 7 days, and avoid repeated freeze/thaw cycles is advised.

③ The 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 on spot.

④ The 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 a serial concentration. The recommended dilution gradient is as follows: 0, 200, 400, 500, 600, 800, 900, 1000 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**200**	**400**	**500**	**600**	**800**	**900**	**1000**
1.0 mmol/L standard (μL)	0	40	80	100	120	160	180	200
Standard diluent (μL)	200	160	120	100	80	40	20	0

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 a 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 10000×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 (E-BC-K318-M).

② Dilution of sample

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. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

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

② If the γ-GT activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).

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.

Operating steps

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.

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.

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

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) 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.

$$\gamma\text{-GT activity (U/L)} = (\triangle A_{sample} - b) \div a \times V_1 \div V_2 \div T \times f$$

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

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.

$$\gamma\text{-GT activity (U/gprot)} = (\triangle A_{sample} - b) \div a \times V_1 \div V_2 \div Cpr \div T \times f$$

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

[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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.50	94.60	207.00
%CV	4.5	4.0	4.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

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

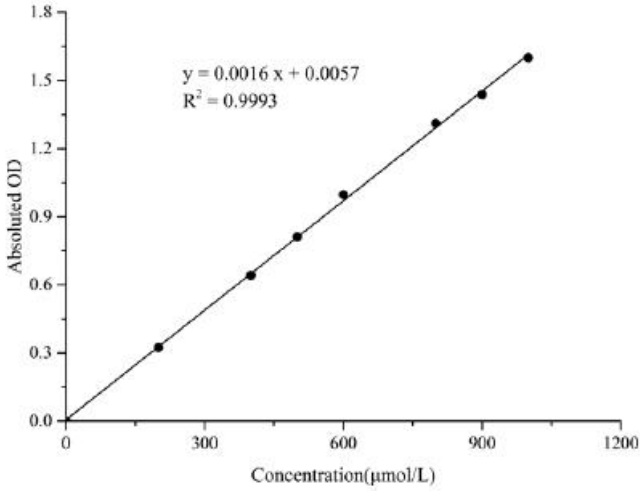
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	200	400	500	600	800	900	1000
OD value	0.042	0.364	0.656	0.844	1.051	1.355	1.485	1.678
	0.042	0.367	0.710	0.862	1.027	1.351	1.474	1.606
Average OD	0.042	0.366	0.683	0.853	1.039	1.353	1.480	1.642
Absoluted OD	0.000	0.324	0.641	0.811	0.997	1.311	1.438	1.600



Appendix II Example Analysis

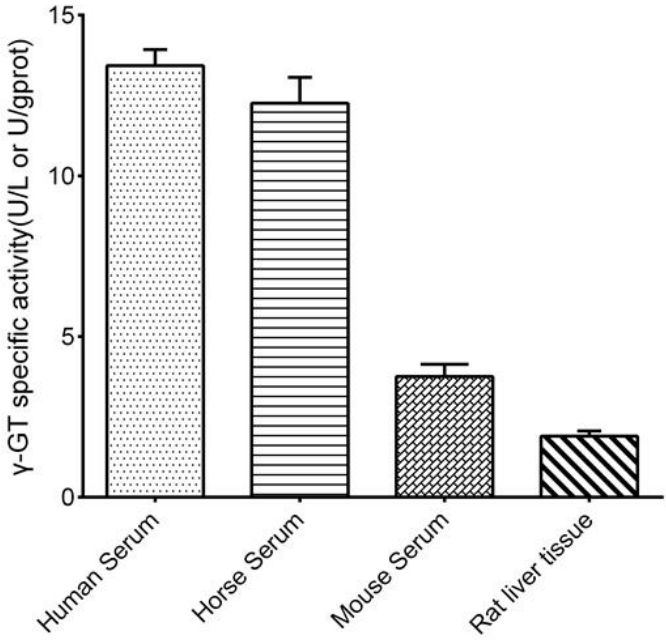
Example analysis：

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

standard curve: y = 0.0016 x + 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:

$$\gamma\text{-GT activity (U/L)} = 0.4 \times (0.048 - 0.0057) \div 0.0016 = 10.575 \text{ U/L}$$

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：



Appendix III Publications

1. Yuan J , Ding L , Han L ,et al.Thermal/ultrasound-triggered release of liposomes loaded with Ganoderma applanatum polysaccharide from microbubbles for enhanced tumour ablation[J].Journal of Controlled Release, 2023, 363(000):17.DOI:10.1016/j.jconrel. 2023.09.030.
2. Guo H , Li Y , Wang S ,et al.Dysfunction of astrocytic glycophagy exacerbates reperfusion injury in ischemic stroke[J].Redox Biology, 2024, 74.DOI:10.1016/j.redox.2024.103234.
3. Guo H , Li Y , Wang S ,et al.Dysfunction of astrocytic glycophagy exacerbates reperfusion injury in ischemic stroke[J].Redox Biology, 2024, 74.DOI:10.1016/j.redox.2024.103234.
4. Hassan H M , Safadi M E , Hayat M F ,et al.Prevention of fenitrothion induced hepatic toxicity by saponarin via modulating TLR4/MYD88, JAK1/STAT3 and NF-κB signaling pathways[J].International Journal of Biochemistry and Cell Biology, 2025, 179.DOI:10.1016/j.biocel.2024.106716.
5. Liu B , Zhang J , Yao S J .San-Huang-Chai-Zhu Formula Ameliorates Liver Injury in Intrahepatic Cholestasis through Suppressing SIRT1/PGC-1α-Regulated Mitochondrial Oxidative Stress[J].Evidence-based complementary and alternative medicine: eCAM, 2022, 2022(Pt.22):ArticleID7832540-ArticleID7832540.DOI:10.1155/2022/7832540.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.