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

Catalog Number EEA029 (96 tests)

Rev 2.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

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

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, 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.

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.

Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Buffer Solution	30 mL
Substrate	Powder ×2 vials
Extracting Solution	50 mL ×2 vails
1.0 mmol/L p-Nitroaniline Standard Solution	1.5 mL
Standard Diluent	10 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water, PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 405 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

The key points of the assay

- The temperature and time of incubation at 37℃ must be accurately.
- If the γ-GT activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (Catalog No. 23227).
- Accurate operation is required when adding liquid to microplate and prevent the formulation of bubbles when adding the liquid to the microplate.
- It is recommended to extend the reaction time of A_2 to 15min for low content samples.

Sample preparation guidelines

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of extracting solution (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for 1 month

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and 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
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.

Preheat the 1.0 mmol/L p-nitroaniline standard solution and standard diluent at 37℃ until clarified before use.

Preparation of substrate application solution

Dissolve a vial of substrate with 3 mL of standard diluent and mix fully. Prepared the solution before use. The prepared solution can be aliquoted and stored at 2-8℃ for 7 days.

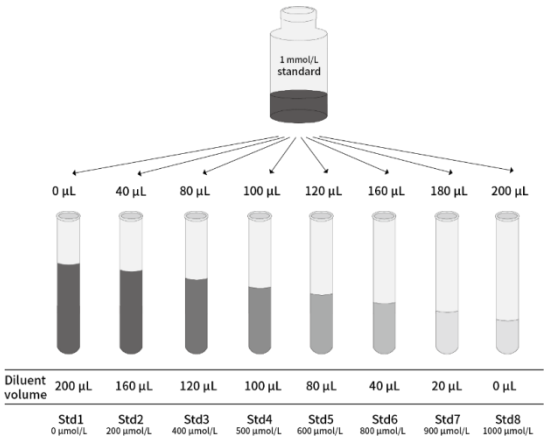
Preparation of reaction working solution

Mix 4 parts of the buffer solution with 1 part of substrate application solution fully. Prepare the fresh solution before use.

Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

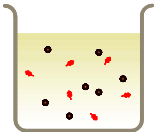
Dilute 1.0 mmol/L p-nitroaniline standard solution with standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 200, 400, 500, 600, 800, 900, 1000 μmol/L.



Preparation of standard working solution

Mix 4 parts of the buffer solution with 1 part of standard solutions fully. Prepare the fresh solution before use."

Assay procedure



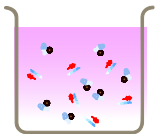
1. Add sample and standard

a. **Standard well:** add 25 μL of distilled water to the corresponding wells.

b. **Sample well:** add 25 μL of sample to the corresponding wells.

c. **Standard well:** add 250 μL of standard working solution with different concentrations to standard wells.

d. **Sample well:** add 250 μL of reaction working solution to sample wells



2. Add substrate

a. Mix fully for 10 s with microplate reader, incubate at 37℃ for 1 min accurately and measure the OD value (A_1) of each well at 405 nm

b. Incubate the microplate at 37℃ for 5 min accurately and measure the OD value (A_2) of each well at 405 nm. $\Delta A=A_2-A_1$. (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

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) 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).

Serum (plasma) and other liquid sample:

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

$$\gamma\text{-GT activity (U/L)} = (\Delta A_{sample} - b) \div a \times V_1 \div V_2 \div T \times f$$
$$= 0.4 \times (\Delta A_{sample} - b) \div a \times f$$

Tissue sample:

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

$$\gamma\text{-GT activity (U/gprot)} = (\Delta A_{sample} - b) \div a \times V_1 \div V_2 \div Cpr \div T \times f$$
$$= 0.4 \times (\Delta A_{sample} - b) \div a \div Cpr \times f$$

[Note]

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)

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

T: reaction time, 5 min

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (gprot/L)

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

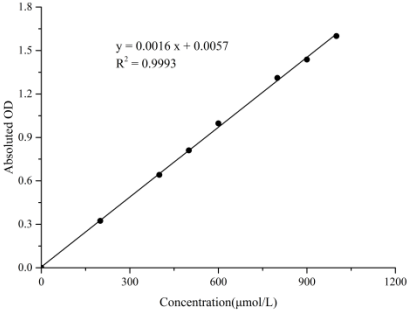
For human serum, take 25 μL of human serum and carry the assay according to the operation steps. The results are as follows: standard curve: y = 0.0016 x + 0.0057, the average OD value of the sample incubation for 1 min (A_1) is 1.111, the average OD value incubation for 5 min (A_2) is 1.159, then $\triangle A_{sample} = A_2 - A_1 = 0.048$, and the calculation result is:

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

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–1000 μmol/L standard.

Concentration (μmol/L)	OD at 405 nm	Standard Curve
1000	1.642	
900	1.480	
800	1.353	
600	1.039	
500	0.853	
400	0.683	
200	0.366	
0	0.042	

- Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

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

CV = Coefficient of Variation

- 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

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum, and plasma samples without dilutions.

Sample	Range (U/L)	Average (U/L)
Human serum	10-16	13.4
Human plasma	9-15	11.6

- Recommended 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												

- 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.

Limited product warranty

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