PRODUCT INFORMATION & MANUAL

gamma-Glutamyl Transferase/GGT Activity Assay Kit (Colorimetric)

NBP3-25925

For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

gamma-Glutamyl Transferase/GGT Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25925

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.88 U/L

Detection range: 0.88-399.4 U/L

Average intra-assay CV (%): 4.2

Average inter-assay CV (%): 6.2

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure gamma-Glutamyl Transferase/GGT (γ-GT) activity in serum, plasma, animal tissue samples.

▲Background

gamma-Glutamyl Transferase/GGT transferase (γ-GT) is widely present in various organs of the human body. It is a key enzyme in the gamma-Glutamyl Transferase/GGT 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 gamma-Glutamyl Transferase/GGT is very low. In patients with acute hepatitis, liver cancer, and obstructive yellow pox, the serum gamma-Glutamyl Transferase/GGT activity is significantly increased. Therefore, the determination of gamma-Glutamyl Transferase/GGT 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.

▲Detection principle

gamma-Glutamyl Transferase/GGT 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 gamma-glutamyl can be calculated according to the changing rate of absorbance value.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	30 mL x 1 vial	2-8°C , 12 months
Reagent 2	Substrate	Powder × 2 vials	2-8°C , 12 months, shading light
Reagent 3	Extracting Solution	50 mL × 2 vials	2-8°C , 12 months
Reagent 4	1.0 mmol/L p-Nitroaniline Standard Solution	1.5 mL x 1 vial	2-8°C , 12 months
Reagent 5	Standard Diluent	10 mL x 1 vial	2-8°C , 12 months
	Microplate	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.			

▲Materials prepared by users

Instruments

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

Reagents

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. The temperature and time of incubation at 37°C must be accurately.
2. If the gamma-Glutamyl Transferase/GGT activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately.
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.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 solution:

Dissolve a vial of reagent 2 with 3 mL of reagent 5 and mix fully. Prepared the solution before use and the prepared solution can be divided into smaller packages and store at 2-8℃ for 7 days.

2. Preparation of reaction working solution:

Mix the reagent 1 and reagent 2 solution at the ratio of 4:1 fully. Prepare the fresh solution before use.

▲Sample preparation

1. Serum (plasma) sample

Detect the serum or plasma samples directly.

2. Tissue sample

Weigh the tissue accurately and add reagent 3 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℃ , then take the supernatant for measurement.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do 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
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 reagent 3.

Assay protocol

▲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.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 1.0 mmol/L p-nitroaniline standard solution with reagent 5 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:

Number	Standard concentrations (μmol/L)	1.0 mmol/ L p-nitroaniline standard (μL)	Reagent 5 (μL)
A	0	0	200
B	200	40	160
C	400	80	120
D	500	100	100
E	600	120	80
F	800	160	40
G	900	180	20
H	1000	200	0

2. Preparation of standard working solution

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

3. 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. $\Delta A=A_2-A_1$.

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.

▲Summary operation table

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

▲Calculation

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.

$$\text{γ-GT activity (U/L)} = (\Delta A_{sample} - b) \div a \times V_{reagent\ 2} \div V_{sample} \div T \times f$$

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

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.

$$\text{γ-GT activity (U/gprot)} = (\Delta A_{sample} - b) \div a \times V_{reagent\ 2} \div V_{sample} \div C_{pr} \div T \times f$$

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

Note:

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}: $A_2 - A_1$.

$V_{reagent\ 2}$: The volume of reagent 2 solution, 50 μL = 5.0×10^{-5} L. (Reaction working solution was mixed reagent 1 and reagent 2 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 x 10-5 L.

T: reaction time, 5 min.

Appendix I Data

▲Example analysis

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

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 $\Delta A=A_2-A_1=0.048$, and the calculation result is:

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