PRODUCT INFORMATION & MANUAL

GPT Activity Assay Kit (Fluorometric)

NBP3-24460

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

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GPT Activity Assay Kit (Fluorometric)

Catalog No: NBP3-24460

Method: Fluorimetric method

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

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.01 U/L

Detection range: 0.01-0.83 U/L

Average intra-assay CV (%): 2.3

Average inter-assay CV (%): 9.8

- ▲ 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 be used to measure alanine aminotransferase (ALT/GPT) activity in animal tissue, serum (plasma) and other liquid samples.

▲Background

Alanine aminotransferase (GPT) is widely found in plasma and various tissues of the body, including liver, kidney, heart and skeletal muscle. GPT is an important pyridoxal phosphate dependent enzyme in the intermediate metabolism of glucose and protein. Clinically, the activity of serum alanine aminotransferase is often used as a marker for alcoholic liver disease, liver cirrhosis and acute viral hepatitis.

▲Detection principle

GPT catalyze the amino conversion reaction between alanine and α-ketoglutaric acid to produce pyruvic acid and glutamic acid. Under the action of pyruvate oxidase, pyruvic acid generates H_2O_2, which reacts with the non-fluorescent substance to form fluorescent substance under the action of peroxidase. The activity of GPT can be calculated by measuring the increase of fluorescence value at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	-20℃ , 12 months
Reagent 2	Probe Solution	0.5 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months
Reagent 4	Substrate Solution	1.2 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 5	100 mmol/L Pyruvate Standard	0.1 mL × 1 vial	-20℃ , 12 months
	Black 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

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Micropipettor, Incubator, Vortex mixer, Water bath, Centrifuge

⚠ 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

Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Pre-assay preparation

▲Reagent preparation

1. The preparation of reagent 3 working solution:
Dissolve a vial of reagent 3 with 1.2 mL of reagent 1 fully and preserve it on ice for use. The prepared reagent 3 working solution can be stored at -20℃ for 1 week.
2. The preparation of 1 mmol/L pyruvic acid standard stock solution:
Take 10 μL of reagent 5 to 990 μL of reagent 1 and mix fully. Prepare the fresh solution before use.
3. The preparation of 50 μmol/L pyruvic acid standard solution:
Dilute 1 mmol/L pyruvic acid standard stock solution with reagent 1 at the ratio of 1: 19 and mix fully. Prepare the fresh solution before use and preserve it on ice for use.
4. Preparation of reaction working solution:
Mix the reagent 1, reagent 2, reagent 3 working solution and reagent 4 at a ratio of 56: 4: 20: 20. Prepare the fresh solution before use and stored with shading light.

▲Sample preparation

1. Serum sample:
Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.
2. Plasma sample:
The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4 ℃ for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed.

Place the plasma on ice for detection.

3. 10% tissue homogenate sample:

Accurately weigh the tissue sample, add 9 times the volume of reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 1500 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲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 (0.01-0.83 U/L).

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

Sample type	Dilution factor
Human serum	10-15
Dog serum	5-10
Rat serum	10-15
10% Mouse heart tissue homogenate	100-120
10% Rat spleen tissue homogenate	10-15
10% Rat liver tissue homogenate	300-500
10% Rat kidney tissue homogenate	100-120
10% Rat lung tissue homogenate	100-120

Note: The diluent is reagent 1.

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

The preparation of standard curve

Dilute 50 μmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 25, 30, 35, 40, 50 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	50 μmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	200
B	10	40	160
C	20	80	120
D	25	100	100
E	30	120	80
F	35	140	60
G	40	160	40
H	50	200	0

The measurement of samples

1) Standard well: add 20 μL of standard with different concentrations into the corresponding well.
Sample well: add 20 μL of sample into the corresponding well.
2) Add 100 μL of reaction working solution to each well.
3) Mix fully with microplate reader for 5 s and stand at room temperature for 3 min.
4) Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, recorded as F_1, and then react at room temperature for 60 min with shading light. The fluorescence intensity of each well was determined under the same wavelength, and recorded as F_2, then $\Delta F = F_2-F_1$ (Note: There is no change in fluorescence value of standard well, plot the standard curve with the fluorescence value of $F_{2(standard)}$).

▲Summary operation table

	Standard well	Sample well
Sample (μL)		20
Standard with different concentrations (μL)	20	
Reaction working solution (μL)	100	100
Mix fully and stand at room temperature for 3 min. Measure the fluorescence intensity of each well, recorded as F_1, and then react at room temperature for 60 min with shading light. The fluorescence intensity of each well was determined, and recorded as F_2, then $\Delta F = F_2-F_1$		

▲Calculation

Plot the standard curve by using fluorescence value (F) 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 F value of sample.

The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

Definition: The amount of enzyme in 1 L of serum or plasma that catalyze the production of 1 μmol pyruvic acid per minute at 25℃ is defined as 1 unit.

$$\text{GPT activity (U/L)} = (\Delta F - b) \div a \div T \times f$$

2. Tissue sample:

Definition: The amount of enzyme in 1 g of tissue protein that catalyze the production of 1 μmol pyruvic acid per minute at 25℃ is defined as 1 unit.

$$\text{GPT activity (U/gprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

Note:

y: $F_{Standard} - F_{Blank}$ (F_{Blank} is the fluorescence value when the standard concentration is 0)

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔF: The absolute fluorescence value of sample, $\Delta F = F_2 - F_1$.

T: The reaction time, 60 min.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Data

▲Example analysis

For rat lung tissue, dilute supernatant of rat lung tissue homogenate for 100 times, take 20 μL of it to corresponding sample wells, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 69.312 x – 148.74, the average F_1 value of the sample is 611, the average F_2 value of the sample is 1577, $\Delta F = F_2-F_1$ =966, the concentration of protein in sample is 2.93 gprot/L, and the calculation result is:

GPT activity (U/gprot)= (966+148.74) ÷ 69.312 ÷60 × 100 ÷2.93=9.15 U/gprot