

Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: JYM017CHS

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact our staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Powder×2	2-8℃
Reagent II	Liquid 3.5 mL×1	2-8℃
Reagent III	Liquid 30 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. Reagent I: An 8 mL brown bottle is provided. Before use, take a reagent I and pour it into the empty 8 mL brown bottle, dissolve it with 2 mL of distilled water, and then rinse the residual reagent with solution. It could be stored at 2-8℃ for 4 weeks. The reagent is a **freeze-dried** reagent, and there may be a large difference or even a small amount of reagent between different bottles. This phenomenon does not affect the use, and the actual quality is the same.

2. Standard: 20 μmol/mL sodium pyruvate.

Product Description:

Glutamic pyruvic transaminase (GPT, EC 2.6.1.2) is widely found in animals, plants, microbes and cultured cells, which is an important enzyme in amino acid metabolism. It catalyzes the transamination of amino acid and keto acid. In addition, GPT activity is very high in mammalian liver cells. GPT is released into the blood when liver cells necrotic, serum GPT activity is significantly increased. Therefore, GPT is recommended as the most sensitive indicator of liver damage by the World Health Organization.

GPT catalyzes the transamination reaction of alanine and α-ketoglutarate to generate pyruvate and glutamic acid; the addition of 2,4-dinitrophenylhydrazine solution not only terminates the above reaction, but also reacts with the carbonyl group in keto acid to form phenylhydrazone pyruvate which shows brownish red in alkaline condition, the activity of GPT enzyme activity can be calculated by measuring the absorbance of 505 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, micro glass cuvette/96 well plate, water bath, desk centrifuge, mortar/homogenizer/cell ultrasonic crusher, adjustable pipette, ice and distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Bacteria or cell: The ratio of bacteria/cell amount (10^6): the volume of extract solution (mL) is 5~10:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 w, work time 3 s, interval 10 s, repeat 30 times). Centrifuge at 3500g for 10 minutes at 4°C, take the supernatant and placed on the ice for test

2. Tissue sample: Add extract solution according to the ratio of tissue mass (g) : extract solution volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL extract solution), after ice bath homogenization, centrifuge at 4°C, 3500g for 10min, take supernatant and placed on the ice for test.

3. Serum (plasma) sample: Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure

- Preheat spectrophotometer/microplate reader for more than 30 min, adjust the wavelength to 505 nm and set spectrophotometer zero with distilled water.
- Prepare standard solution:Dilute 20 μmol / mL sodium pyruvate solution with distilled water to 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0 μmol / mL. (**0 is a blank tube**).
- Operation table:

Reagent name (μL)	Test tube(T)	Contract tube(C)	Standard tube(S)
Sample	5	-	-
Reagent I	25	25	-
Standard	-	-	30
Mixed thoroughly, reaction at 37 °C for 30 min			
Reagent II	25	25	25
Sample		5	
Mixed thoroughly, reaction at 37 °C for 20 min			
Reagent III	240	240	240
Mixed thoroughly, stand 10 min at room temperature and then take 200 μL to micro glass cuvette/96 well plate, detect absorbance at 505 nm, and record them as A_T , A_C , A_S and A_B (It is 0$\mu\text{mol/mL}$ standard tube), and calculate $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$. Each test tube should be provided with one control tube. Standard curve only need to be measured 1-2 times.			

III. Calculation:

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation $y = kx + b$, and bring ΔA_T into the equation to get x ($\mu\text{mol/mL}$).

2. Calculation of GPT activity

(1) Calculate by sample mass

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of pyruvate per hour every g sample mass.

$$\text{GPT activity (U/g mass)} = x \times (V_S + V_{RI}) \div (W \times V_S \div V_E) \div T \times F = 12x \div W \times F$$

(2) Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of pyruvate per hour every mg protein.

$$\text{GPT activity (U/mg prot)} = x \times (V_S + V_{RI}) \div (C_{pr} \times V_S) \div T \times F = 12x \div C_{pr} \times F$$

(3) Calculate by liquid volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of pyruvate per hour every mL liquid.

$$\text{GPT activity (U/mL)} = x \times (V_S + V_{RI}) \div V_S \div T \times F = 12x \times F$$

(4) Calculate by the number of bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of pyruvate per hour every 10^6 cells.

$$\text{GPT activity (U/10}^6 \text{ cell)} = x \times (V_S + V_{RI}) \div (N \times V_S \div V_E) \div T \times F = 12x \div N \times F$$

V_S : The sample volume, 0.005 mL;

V_{RI} : Reagent I volume, 0.025 mL;

V_E : Extract solution volume, 1 mL;

W : Sample mass, g;

T : Reaction time, 0.5 h;

C_{pr} : Sample protein concentration, mg/mL;

N : The numbers of cells or bacteria, count by 10^6 ;

F : Dilution multiple

Experimental example:

1. Take 0.103g rabbit liver and add 1mL of extract solution for ice bath homogenization, take supernatant and then follow the determination steps. After determination with 96 well plate, calculate, $\Delta A_T = A_T - A_C = 0.934 - 0.391 = 0.543$, bring into standard curve: $y = 0.7915x - 0.0107$, $R^2 = 0.9995$, obtained $x = 0.700$, calculate GPT activity by sample mass:

$$\text{GPT activity (U/g mass)} = 12x \div W \times F = 81.56 \text{ U/g mass}$$

2. Take HEB 6.6×10^6 cells and add 1mL of extract solution for ice bath homogenization, take supernatant and then follow the determination steps. After determination with 96 well plate, calculate, $\Delta A_T = A_T - A_C = 0.361 - 0.325 = 0.036$, bring into standard curve: $y = 0.7915x - 0.0107$, $R^2 = 0.9995$, obtained $x = 0.059$, calculate GPT activity by sample mass:

$$\text{GPT activity (U/10}^6 \text{ cell)} = 12x \div N \times F = 0.107 \text{ U/10}^6 \text{ cell}$$