Glutamic-oxalacetic Transaminase (GOT) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: JYM012CHS

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:

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.

Standard: 20 μmol/mL sodium pyruvate.

Product Description:

Glutamic-oxalacetic transaminase (GOT, EC 2.6.1.1) is widely found in animals, plants, microbes and cultured cells. It catalyzes the reversal of amino reactions and is an important enzyme in amino acid metabolism. In addition, GOT is the highest in cardiomyocytes and is commonly used as an assisted examination of myocardial infarction and myocarditis in clinical practice. The serum concentration of liver damage can also be increased.

GOT catalyze α-ketoglutaric acid react with aspartate to produce glutamic acid and oxaloacetic acid. Oxaloacetic acid is further decarboxylated to form pyruvate, pyruvate can react with 2, 4-dinitrophenylhydrazine to produce 2,4-dinitrophenylhydrazone, which shows brownish red in alkaline condition, the activity of GOT enzyme activity can be calculated by measuring the absorbance of 505 nm.

Reagent and Equipments 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℃, 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℃, 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

1. Preheat spectrophotometer/microplate reader for more than 30 min, adjust the wavelength to 505 nm and set spectrophotometer zero with distilled water.
2. 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**).
3. 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 ℃ for 30 min			
Reagent II	25	25	25
Sample	-	5	-
Mixed thoroughly, reaction at 37 ℃ 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 **0μmol/mL s**tandard tube), and calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. 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 (μmol/mL).

2. Calculation of GOT 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.

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

(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.

GOT activity(U/mg prot)=x×(Vs+V_{RI})÷(Cpr×Vs)÷T×F=12x÷Cpr×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.

GOT activity(U/mL）=x×(Vs+V_{RI})÷Vs÷T×F=12x×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.

GOT activity(U/10^6 cell)=x×(Vs+V_{RI})÷(N×Vs÷V_E)÷T×F= 12x÷N×F

Vs: 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;

Cpr: 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, A_T= 0.905, A_C=0.429, bring into standard curve: y=0.7915x-0.0107, R^2=0.9995, calculate $\Delta A_T=A_T-A_C$= 0.476, obtained x=0.615, calculate GOT activity by sample mass:
   GOT activity (U/g mass) = 12x÷W×F=71.65 U/g mass
2. Take bovine serum and follow the determination steps. After determination with 96 well plate, A_T= 0.385, A_C=0.305, bring into standard curve: y=0.7915x-0.0107, R^2=0.9995, calculate $\Delta A_T=A_T-A_C$= 0.080, obtained x=0.115, calculate GOT activity by sample volume:
   GOT activity (U/g mass) = 12x×F=1.38 U/mL
3. Take NCTC1469 10×10^6 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, A_T= 0.439, A_C=0.261, bring into standard curve: y=0.7915x-0.0107, R^2=0.9995, calculate $\Delta A_T=A_T-A_C$= 0.232, obtained x=0.307, calculate GOT activity by the number of cells:
   GOT activity (U/10^6cell) = 12x÷N×F=0.368 U/10^6 cell