

Glutamic-oxalacetic Transaminase (GOT) Activity Assay Kit

Detection equipment: Spectrophotometer

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

Cat No: AK0421

Size: 50T/24S

Components:

Extract solution: 30 mL×1, store at 4°C;

Reagent I: Powder×2, store at 4°C; Add 4 mL distilled water into every tube before use. Dissolve the reagent when it will be used.

Reagent II: 8 mL×1, store at 4°C;

Reagent III: 80 mL×1, store at 4°C;

Standard: 1 mL×1, 20 μmol/mL sodium pyruvate, store at 4°C;

Product Description:

GOT 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, water bath, desk centrifuge, transferpeltor, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation:

1. Cells or microorganism: collect sample to centrifuge tube, discard supernatant, accordance sample : extract solution=5 million : 1. Ultrasonic smash cells or microorganism(power 20%, ultrasonic 3s, interval 10s, repeat 30 times). 3500 g centrifuge at 4°C for 10 min, take supernatant on ice is ready for test.
2. Tissue: add 1 mL extract solution to 0.1 g tissue homogenate on ice, 3500 g centrifuge at 4°C for 10 min, take supernatant on ice is ready for test.
3. Serum: directly detect.

II. Detect procedure:

1. Preheat spectrophotometer for 30 min, adjust wavelength to 505 nm, set zero with distilled water.
2. Standard curve detection

Firstly, the standard is diluted to 2 $\mu\text{mol/mL}$ with distilled water, and the corresponding concentration standard tube is obtained by mixing the standard and reagent according to the table below:

Standard (μL)	Reagent I (μL)	Standard tube ($\mu\text{mol/mL}$)
120	0	2
90	30	1.5
60	60	1
45	75	0.8
24	96	0.4
12	108	0.2
6	114	0.1
3	117	0.05
0	120	0

3. Add following reagents to centrifuge tube

Reagent name (μL)	Test tube (At)	Control tube (Ac)	Standard tube (As)
Sample	20		
Reagent I	100	100	
Standard solution			120

Mix thoroughly, incubate at 37°C(mammal)or 25°C(other animal)for 30 min.			
Reagent II	100	100	100
Sample		20	
Mix thoroughly, incubate at 37°C(mammal)or 25°C(other animal)for 20 min.			
Reagent III	1000	1000	1000
Mix thoroughly, incubate at room temperature for 10 min, detect the absorbance at 505 nm wavelength.			

Note: 0 μmol/mL standard tube as the blank tube.

III. Calculation

1. Standard curve

Using standard solution concentration as x axis, $\Delta A(A_s - A_b)$ as y axis, the equation $y = kx + b$ is obtained. $A_t - A_c$ is brought into the equation to calculate x value.

2. GOT activity calculation

A. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol pyruvic acid in the reaction system per minute every gram sample.

$$\text{GOT(U/g weight)} = x \times (V_s + V_{RI}) \div (W \times V_s \div V_{ST}) \div T = 12x \div W$$

B. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol pyruvic acid in the reaction system per minute every mg protein.

$$\text{GOT(U/mg prot)} = x \times (V_s + V_{RI}) \div (C_{pr} \times V_s) \div T = 12x \div C_{pr}$$

C. Serum volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol pyruvic acid in the reaction system per minute every mL serum.

$$\text{GOT(U/mL)} = x \times (V_s + V_{RI}) \div V_s \div T = 12x$$

D. Cells amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol pyruvic acid in the reaction system per minute every 10⁴ cells.

$$\text{GOT(U/10}^4 \text{ cell)}=x \times (V_S + V_{RI}) \div (V_S \div V_{ST} \times 500) \div T = 0.024x$$

V_S : Sample volume, 0.02 mL;

V_{RI} : Reagent I volume, 0.1 mL;

V_{ST} : Extract solution volume, 1 mL;

W : Sample weight, g;

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

T : Reaction time, 0.5 h;

500: Cells or germ amount, 5 million.

Related products:

AK0582/AK0581 Cysteine(Cys) Content Assay Kit

AK0417/AK0416 Glutamic Acid(Glu) Content Assay Kit

AK0572/AK0571 Hydroxyproline(HYP) Content Assay Kit

Experimental example:

1. Take 0.1 g rabbit liver to 1 ml extract solution, grinding and operate as the procedure after taking the supernatant, $A_t=0.782$, $A_c=0.488$, $\Delta A=A_t-A_c=0.782-0.488=0.294$, calculate by standard curve: $y=0.4767x+0.0611$, $x=(0.294-0.0611) \div 0.4767=0.489$, calculate content by sample weight: $\text{GOT (U/g weight)}=12x \div W=12 \times 0.489 \div 0.1=56.68 \text{ U/g weight}$.

References:

[1] Yong Li, Fengjun Cao, Mingxing Li, et al. Hydroxychloroquine induced lung cancer suppression by enhancing chemo-sensitization and promoting the transition of M2-TAMs to M1-like macrophages. Journal of Experimental & Clinical Cancer Research. October 2018;(IF5.646)

[2] Poopal R K, Zhang J, Zhao R, et al. Biochemical and behavior effects induced by diheptyl phthalate (DHpP) and Diisodecyl phthalate (DIDP) exposed to zebrafish[J]. Chemosphere, 2020: 126498.