Glutamic-oxalacetic Transaminase (GOT) Activity Assay Kit

Detection equipment: Spectrophotometer/microplate reader

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

Cat No: NA0501

Size: 100T/48S

Components:

Extract solution: Liquid 60 mL×1, store at 4℃;

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

Reagent II: Liquid 3.5 mL×1, store at 4℃;

Reagent III: Liquid 30 mL×1, store at 4℃;

Standard: Liquid 1 mL×1, 20 μmol/mL sodium pyruvate, store at 4℃;

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/microplate reader, water bath, desk centrifuge, transferpettor, micro glass cuvette/96 well flat-bottom plate, 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℃ for 10 min, take supernatant on ice is ready for test.

2. Tissue

Add 1mL extract solution to 0.1 g tissue homogenate on ice, 3500 g centrifuge at 4℃ for 10 min, take supernatant on ice is ready for test.

3. Serum: Directly detect.

II. Detect procedure:

1. Preheat spectrophotometer or microplate reader for 30 min, adjust wavelength to 505 nm, set zero with distilled water.

2. Standard curve detection

Firstly, the standard was diluted to 2 μmol/mL with distilled water, and the corresponding concentration standard tube was obtained by mixing the standard and reagent according to the table below:

Standard (μL)	Reagent I (μL)	Standard tube (μmol/mL)
22.5	7.5	1.5
15	15	1
12	18	0.8
6	24	0.4
3	27	0.2
1.5	28.5	0.1
0.75	29.25	0.05
0	30	0

3. Add following reagents to centrifuge tube

Reagent name (μL)	Test tube (At)	Control tube (Ac)	Standard tube (As)
Sample	5		
Reagent I	25	25	

Standard solution			30
Mix thoroughly, incubate at 37℃(mammal)or 25℃ (other animal) for 30 min.			
Reagent II	25	25	25
Sample		5	
Mix thoroughly, incubate at 37℃(mammal)or 25℃ (other animal) for 20 min.			
Reagent III	240	240	240
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, ΔA(As-Ab) as y axis, the equation y=kx+b is obtained. (At-Ac) 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.

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

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.

$GOT(U/mg\ prot)=x\times(Vs+V_{RI})\div(Cpr\times Vs)\div T=12x\div Cpr$

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.

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

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 10^4 cells.

GOT(U/10^4 cell)=x×(Vs+V_{RI})÷(Vs÷V_{ST}×500)÷T=0.024x

V_S: Sample volume, 0.005 mL;

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

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

W: Sample weight, g;

Cpr: Sample protein concentration, mg/mL;

T: Reaction time, 0.5 h;

500: Cells or germ amount, 5 million.

Related products：

NA0856/NA0614 Cysteine(Cys) Content Assay Kit

NA0741/NA0499 Glutamic Acid(Glu) Content Assay Kit

NA0849/NA0607 Hydroxyproline(HYP) Content Assay Kit

Experimental example:

1. Take 0.1g rabbit liver to 1ml extract solution, grinding and operate as the procedure after taking the supernatant, At=0.451, Ac=0.281, ΔA=At-Ac= 0.451-0.281=0.170, calculate by standard curve: y=0.2817x+0.0211, x=（0.170-0.0211）÷0.2817=0.53, calculate content by sample weight: GOT（U/g weight)= 12x÷W=12×0.53÷0.1=63.6 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.