Glutamic-pyruvic transaminase（GPT）Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC1550

Size: 50T/24S

Components:

Extract solution: 30 mL×1. Storage at 4℃；

Reagent I: Powder×2. Storage at 4℃. At the same time, an 8 mL brown bottle is provided; before use, take a Reagent I and pour it into an empty bottle, dissolve it with 4 mL of distilled water, and then rinse the residual reagent with solution;

Reagent II: 8 mL×1. Storage at 4℃.

Reagent III: 80 mL×1. Storage at 4℃.

Standard: 1 mL×1, 20 μmol/mL sodium pyruvate. Storage at 4℃.

Reagents and Equipment Required but Not Provided:

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

Product Description:

GPT 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 increases Into phenylpyrene pyruvate; which shows brownish red in alkaline condition, the activity of GOT enzyme activity can be calculated by measuring the absorbance of 505 nm.

Procedure:

I. Sample preparation:

A. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. Suggested 5 million with 1 mL of Extract Solution. Use ultrasonication to splitting bacteria or cells (powder 20%, work time 3s，interval 10s，repeat for 30 times). centrifuge at 3500×g, 4℃ for 10 min. Supernatant is used for test.

B. Tissue

Accordance ratio tissue weight (g) : Extract Solution volume (mL)=1：5~10. Suggested 0.1 g of tissue with 1 mL of Extract Solution. Fully grinding on ice, centrifuge at 3500×g, 4℃ for 10 min. Supernatant is used for test.

C. Serum (plasma) sample：Detect sample directly.

II. Determination procedure

(1) Preheat the spectrophotometer 30 min, adjust the wavelength to 505 nm and set zero with distilled water.

(2) Prepare standard solution

First, dilute the standard to 2 μmol/mL. The standard tubes of different concentrations are obtained by the following table operation.

Standard tube（μL）	Reagent I（μL）	Concentration of standard tube（μmol/mL）
90	30	1.5
60	60	1
45	75	0.8
36	84	0.6
24	96	0.4
12	108	0.2
6	114	0.1
3	117	0.05
0	120	0

(3) Add the following reagents to the EP tube

Reagent name（μL）	Test tube	Contract tube	Standard tube
Sample	20		
Reagent I	100	100	
Standard			120

Mixed thoroughly，37℃(mammal) or 25℃(Other species) water bath for 30 min

Reagent II	100	100	100
Sample		20	

Mixed thoroughly，37℃(mammal) or 25℃(Other species) water bath for 20 min

Reagent III	1000	1000	1000

Mix thoroughly, react 10 min at room temperature and then detect the absorbance value of each tube at 540 nm.

Note: 0 μmol/mL standard tube is blank tube.

III. Calculation

1. Standard curve

The concentration of the standard solution as the X-axis, the ΔA ($A_{\text{standard tube}}$ -$A_{\text{blank tube}}$) as the Y-axis, obtain a standard curve y=kx+b. Take (A_{test} -A_{contract}) into the equation to find the x value.

2. Calculation

A. Sample weight:

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 weight.

GPT (U/g weight)=$x\times(Vs+V_{\text{Reagent I}})\div(W\times Vs\div Vsv)\div T=12x\div W$.

B. 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.

GPT (U/mg prot)=$x\times(Vs+V_{\text{Reagent I}})\div(Cpr\times Vs)\div T=12x\div Cpr$.

C. Serum (plasma) sample

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 serum (plasma).

GPT (U/mL)=x×(Vs+$V_{Reagent\ I}$)÷Vs÷T=12x.

Vs: Sample volume, 0.02 mL；

$V_{Reagent\ I}$: Reagent I volume, 0.1 mL；

Vsv: Extraction volume, 1 mL;

W: Sample weight, g;

T: Reaction time，0.5 h；

Cpr: Sample protein concentration, mg/mL;

500: The numbers of cells or bacteria, 5 million cells.

Related products：

BC0180/BC0185 Cysteine(Cys) Content Assay Kit

BC1580/BC1585 Glutamic Acid(Glu) Content Assay Kit

BC0250/BC0255 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, A_{test}=0.701, $A_{contract}$=0.271, ΔA=A_{test}-$A_{contract}$= 0.701-0.271=0.430, calculate by standard curve: y=0.2796x+0.0476, x=（0.430-0.0476）÷0.2796=1.368, calculate content by sample weight: GPT（U/g weight)= 12x÷W=12×1.368÷0.1=164.16 U/g weight.
2. Take 0.1g rabbit serum, operate directly, test and calculate A_{test}=0.307, $A_{contract}$=0.247, ΔA=A_{test}-$A_{contract}$= 0.307-0.247=0.06, calculate by standard curve: y=0.2796x+0.0476， x=（0.06-0.0476）÷0.2796=0.044, calculate content by serum volumn: GPT（U/mL)= 12x=12×0.044=0.528 U/ mL.

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.