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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: AK0422

Size: 100T/48S

Components:

Extract solution: 60mL×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 2 mL of distilled water, and then rinse the residual reagent with solution;

Reagent Ⅱ: 3.5 mL×1. Storage at 4℃ .

Reagent Ⅲ: 30 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/microplate reader, micro glass cuvette/96 well flat-bottom plate, water bath, 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:

1. Extraction

A. Bacteria or cells

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

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

2. Determination procedure

(1) Preheat the spectrophotometer/microplate reader 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）
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 the following reagents to the EP tube/96 well flat-bottom plate

Reagent name（μL）	Test tube	Contract tube	Standard tube
Sample	5		
Reagent I	25	25	
Standard			30

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

Reagent Ⅱ	25	25	25
Sample		5	

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

Reagent Ⅲ	240	240	240

Mixed thoroughly, react 10 min at room temperature and then detect the absorbance value of each tube at

505 nm.

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

3. Calculation

1. Set 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.

D. The number of 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 10^4 cells.

GPT (U/mg prot)=x×(Vs+$V_{Reagent\ I}$)÷(500×Vs÷Vsv)÷T=12x.

Vs: The sample volume, 0.005 mL;

$V_{Reagent\ I}$, 0.025 mL;

Vsv: The 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:

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. 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.

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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.