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°C;

Reagent I: Powder×2. Storage at 4°C. 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; Preserved at 2-8 °C for 4 weeks; the reagent is a freeze-dried reagent, and there may be a large difference in the amount of reagents observed by the naked eye or even a small amount. This phenomenon does not affect the use and the actual quality is the same;

Reagent II: 8 mL×1. Storage at 4°C.

Reagent III: 80 mL×1. Storage at 4°C.

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

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°C for 10 min. Supernatant is used for test.

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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

Dilute the 20 μmol/mL standard 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0 μmol/mL（**0 is the blank tube**）.

(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. Recorded as A_{test}, $A_{contract}$, $A_{standard\ tube}$ and $A_{blank\ tube}$ (i.e., 0 μmol / mL standard). The standard curve only needs to be done 1-2 times.

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 ($A_{standard\ tube}$ -$A_{blank\ tube}$) 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)=$x\times(Vs+V_{Reagent\ I})\div(W\times Vs\div Vsv)\div T=12x\div W$.

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)=$x\times(Vs+V_{Reagent\ I})\div(Cpr\times Vs)\div T=12x\div Cpr$.

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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 $_{Reagent\ I}$)÷(N×Vs÷Vsv)÷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;
N: The numbers of cells or bacteria, 10^4 cell as a unit

Experimental example:

1. Take 0.1g rabbit liver to 1ml extract solution, grinding and operate as the procedure after taking the supernatant, test with 1 mL glass cuvette and calculate, $\Delta A=A_{test}-A_{contract}=0.980-0.433=0.547$, calculate by standard curve: y=1.1563x+0.0024, R^2=0.9992, x=0.471, calculate content by sample weight:
   GPT（U/g weight)= 12x÷W×F =109.75 U/g weight.
2. Take 6.6×10^6 HEB cells to 1ml extract solution, grinding and operate as the procedure after taking the supernatant, test with 1 mL glass cuvette and calculate, $\Delta A=A_{test}-A_{contract}=$ 0.48-0.424=0.056, calculate by standard curve: y=1.1563x+0.0024, R^2=0.9992, x=0.046, calculate content by cell number:
   GOT（U/10^6 cell）=12x÷N×F =0.084 U/10^6 cell

Recent Protect Citations:

[1] Li Y, Fu Y, Zhang Y, Duan B, Zhao Y, Shang M, Cheng Y, Zhang K, Yu Q, Wang T. Nuclear Fructose-1,6-Bisphosphate Inhibits Tumor Growth and Sensitizes Chemotherapy by Targeting HMGB1. Adv Sci (Weinh). 2023 Mar;10(7):e2203528. doi: 10.1002/advs.202203528. Epub 2023 Jan 15. PMID: 36642839; PMCID: PMC9982576.

[2] Yue C, Li D, Fan S, Tao F, Yu Y, Lu W, Chen Q, Yuan A, Wu J, Zhao G, Dong H, Hu Y. Long-term and liver-selected ginsenoside C-K nanoparticles retard NAFLD progression by restoring lipid homeostasis. Biomaterials. 2023 Oct;301:122291. doi: 10.1016/j.biomaterials.2023.122291. Epub 2023 Aug 20. PMID: 37619263.

[3] Tian D, Yu Y, Yu Y, Lu L, Tong D, Zhang W, Zhang X, Shi W, Liu G. Tris(2-chloroethyl) Phosphate Exerts Hepatotoxic Impacts on Zebrafish by Disrupting Hypothalamic-Pituitary-Thyroid and Gut-Liver Axes. Environ Sci Technol. 2023 Jun 20;57(24):9043-9054. doi: 10.1021/acs.est.3c01631. Epub 2023 Jun 5. PMID: 37276532.

[4] Xiang J, Wang SW, Tao Y, Ye JZ, Liang Y, Peng XX, Yang LF, Li H. A glucose-mediated antibiotic resistance metabolic flux from glycolysis, the pyruvate cycle, and glutamate metabolism to purine metabolism. Front Microbiol. 2023 Oct 17;14:1267729. doi: 10.3389/fmicb.2023.1267729. PMID: 37915850; PMCID: PMC10616527.

[5] Pan L, Yang L, Yi Z, Zhang W, Gong J. TBK1 participates in glutaminolysis by mediating the phosphorylation of RIPK3 to promote endotoxin tolerance. Mol Immunol. 2022 Jul;147:101-114. doi: 10.1016/j.molimm.2022.04.009. Epub 2022 May 6. PMID: 35533409.

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.

Related products:

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BC1580/BC1585	Glutamic Acid(Glu) Content Assay Kit
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