# Glutamic-oxalacetic Transaminase (GOT) Activity Assay Kit

**Detection equipment:** Spectrophotometer

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

**Cat No:** BC1560

**Size:** 50T/24S

**Components:**

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

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

Reagent III: 80 mL×1, store at 4℃;

Standard: 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, water bath, desk centrifuge, transferpettor, 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℃ 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℃ 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

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 following reagents to centrifuge 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 At, Ac, As and Ab (i.e., 0 μmol / mL standard). The standard curve only needs to be done 1-2 times.

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, 0.02 mL;
$V_{RI}$: Reagent I volume, 0.1 mL;
VST: 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.

**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, ΔA=$A_{test}$-$A_{contract}$=0.955-0.501=0.454, calculate by standard curve: y=1.1563x+0.0024, $R^2$=0.9992, x=391, calculate content by sample weight:
   GPT（U/g weight)= 12x÷W×F =91.11 U/g weight.
2. Take 10×$10^6$ NCTC1469 cells to 1ml extract solution, grinding and operate as the procedure after taking the supernatant, test with 1 mL glass cuvette and calculate, ΔA=$A_{test}$-$A_{contract}$= 0.732-0.381=0.351, calculate by standard curve: y=1.1563x+0.0024, $R^2$=0.9992, x=0.301, calculate content by cell number:
   GOT（U/$10^6$ cell）=12x÷N×F =0.361 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] 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.

[3] Ji M, Su L, Liu L, Zhuang M, Xiao J, Guan Y, Zhu S, Ma L, Pu H. CaMKII regulates the proteins TPM1 and MYOM2 and promotes diacetylmorphine-induced abnormal cardiac

rhythms. Sci Rep. 2023 Apr 10;13(1):5827. doi: 10.1038/s41598-023-32941-6. PMID: 37037889; PMCID: PMC10085977.

[4] 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.

[5] Bao Z, Guo C, Chen Y, Li C, Lei T, Zhou S, Qi D, Xiang Z. Fatty acid metabolization and insulin regulation prevent liver injury from lipid accumulation in Himalayan marmots. Cell Rep. 2023 Jul 25;42(7):112718. doi: 10.1016/j.celrep.2023.112718. Epub 2023 Jun 28. PMID: 37384524.

**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.

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