（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1300-M

Specification：48T(48 samples)/96T(96 samples)

Measuring instrument：Microplate reader(340 nm)

Detection range：10-1000 U/L

Elabscience® Alanine Aminotransferase (ALT/GPT) Activity Colorimetric Assay Kit (Alanine Substrate Method)

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tel: 1-832-243-6086

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Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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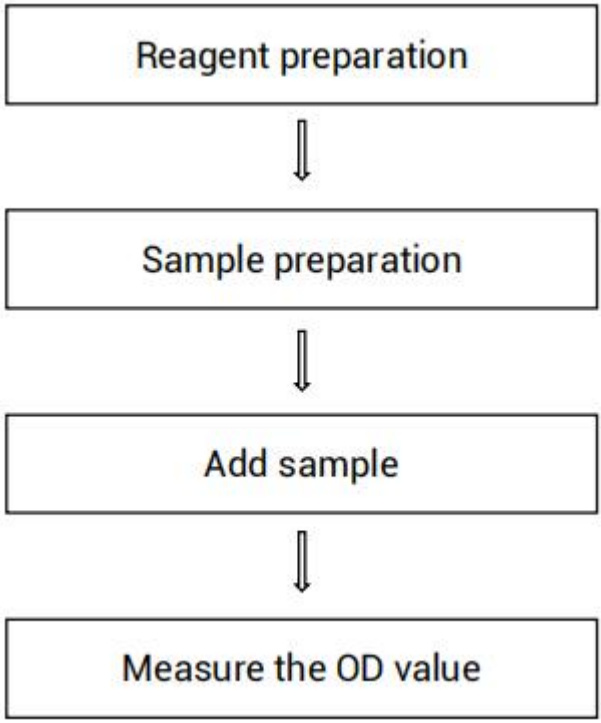
Assay summary



Intended use

This kit can be used to measure alanine aminotransferase (ALT/GPT) activity in serum, plasma, animal tissue and cell samples.

Detection principle

Alanine Aminotransferase (ALT/GPT), is one of the most commonly used and sensitive indicators for liver function testing. Under normal circumstances, the concentration of alanine aminotransferase in human blood is very low. Only when liver cells are damaged or the permeability of the liver cell membrane increases, will the concentration of alanine aminotransferase in the serum increase.

Alanine aminotransferase catalyzes the formation of pyruvic acid from alanine. Pyruvic acid, under the action of dehydrogenase, consumes NADH, causing a decrease in the absorbance at 340 nm. The activity of alanine aminotransferase is determined by measuring the rate of the decrease in absorbance at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Reagent	11 mL × 1 vial	22 mL × 1 vial	2-8°C, 12 months shading light
Reagent 2	Substrate Solution	2.8 mL × 1 vial	5.6 mL × 1 vial	2-8°C, 12 months shading light
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

Reagents:

PBS(0.01 M，pH 7.4)

Reagent preparation

Equilibrate all reagents to 25°C before use.

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL PBS(0.01 M，pH 7.4) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 8 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL PBS(0.01 M，pH 7.4) with a ultrasonic cell disruptor at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 8 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	5-10
10% Mouse kidney tissue homogenate	1
1×10^6 K562 cells	1
Human serum	1
Human plasma	1
Mouse serum	1

Note: The diluent is PBS(0.01 M，pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The ALT/GPT activity in human serum and plasma samples is usually relatively low. The incubation time can be extended from 3-5 min when detecting such samples. Correspondingly, the reaction time in the calculation formula should be modified to 5 min.

② The A_1 value needs to be greater than 0.7 and the A_2 value needs to be greater than 0.2. To avoid enzyme activity exceeding the detection limit, the sample needs to be diluted if is not within this range.

Operating steps

① Sample well: add 10 μL of sample into sample wells.

② Add 200 μL of enzyme reagent into sample wells.

③ Mix fully and incubate at 37°C for 5 min.

④ Add 50 μL of substrate solution into sample wells.

⑤ Mix fully and incubate at 37°C for 1 min.

⑥ Measure the OD values of each well at 340 nm with microplate reader, recorded as A_1.

⑦ Incubate at 37°C for 3 min immediately, measure the OD value of each well at 340 nm with microplate reader, recorded as A_2. $\Delta A = A_1 - A_2$.

Calculation

The sample:

1. Serum or plasma samples:

Definition: The amount of 1 L serum or plasma per 1 min that consume 1 μmol of NADH at 37 °C is defined as 1 unit.

$$\underset{(U/L)}{\text{ALT/GPT activity}} = \Delta A \times \frac{V_2}{\varepsilon \times d} \times 10\text{^}6* \div T \div V_1 \times f = \Delta A \times 1935* \times f$$

2. Tissue or cell samples:

Definition: The amount of 1 g animal tissue or cell protein per 1 min that consume1 μmol of NADH at 37 °C is defined as 1 unit.

$$\underset{(U/gprot)}{\text{ALT/GPT activity}} = \Delta A \times \frac{V_2}{\varepsilon \times d} \times 10\text{^}6* \div T \div (C_{pr} \times V_1) \times f$$

$$= \Delta A \times 1935* \div C_{pr} \times f$$

3. Cell samples:

Definition: The amount of 1×10^6 cells per 1 min that consume 1 μmol of NADH at 37 °C is defined as 1 unit.

$$\underset{(U/10\text{^}6)}{\text{ALT/GPT activity}} = \Delta A \times \frac{V_2}{\varepsilon \times d} \times 10\text{^}6* \div T \div (n \times \frac{V_1}{V_3}) \times f = \Delta A \times 0.4** \div n \times f$$

[Note]:

ΔA: $A_1 - A_2$.

ε: The molar extinction coefficient at 340 nm, 6220 L/moL/cm.

d: Optical path, 0.72 cm.

10^6*:1 mol = 1×10^6 μmol.

V_1: The volume of sample, 10 μL = 1 × 10^-5 L.

V_2: The volume of reaction system, 260 μL = 2.6 × 10^-4 L.

V_3: The volume of PBS(0.01 M，pH 7.4) added when homogenizing the cell sample, 200 μL = 2 × 10^-4 L.

n: The amount of cell/1×10^6.

T: Reaction time, 3 min.

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

1935*: Simplified value 1.

0.4**: Simplified value 2.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	123	235	449
%CV	1.3	0.9	1.8

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	157	302	594
%CV	3.1	3.1	6.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	500	510	523
Observed Conc. (U/L)	499	487	550
Recovery rate (%)	100	95	105

Sensitivity

The analytical sensitivity of the assay is 10 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

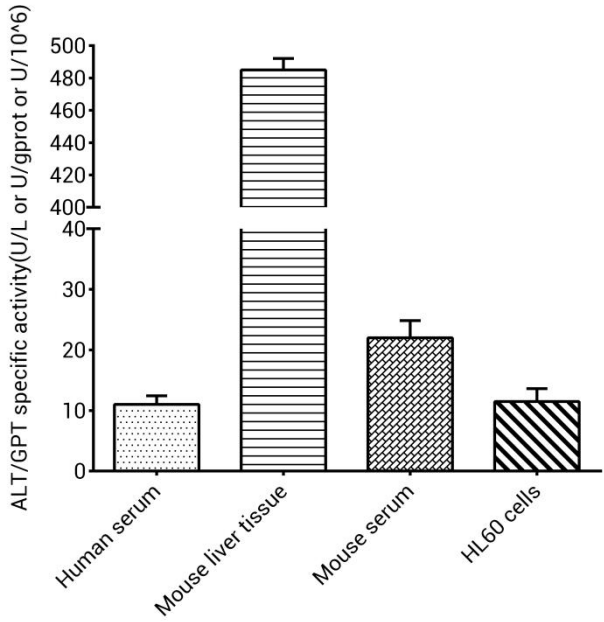
Example analysis：

Take 10 μL of 10% mouse liver tissue homogenate which dilute for 10 times and carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample well is 0.705, the A_2 of the sample well is 0.438, the concentration of protein in sample is 10.8 gprot/L) and the calculation result is:

ALT/GPT activity (U/gprot) = (0.705 - 0.438) × 1935 ÷ 10.8 × 10 = 478 U/gprot

Detect human serum, 10% mouse liver tissue homogenate (the concentration of protein is 10.8 gprot/L, dilute for 10 times), mouse serum, 1×10^6 HL60 cells, according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.