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

Catalog No：E-BC-K235-M

Specification：48T(18 samples)/96T(42 samples)/ 500Assays (244 samples)

Measuring instrument：Microplate reader（500-520 nm）

Detection range：0.75-72.3 IU/L

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

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
Tell: 1-832-243-6086
Fax: 1-832-243-6017
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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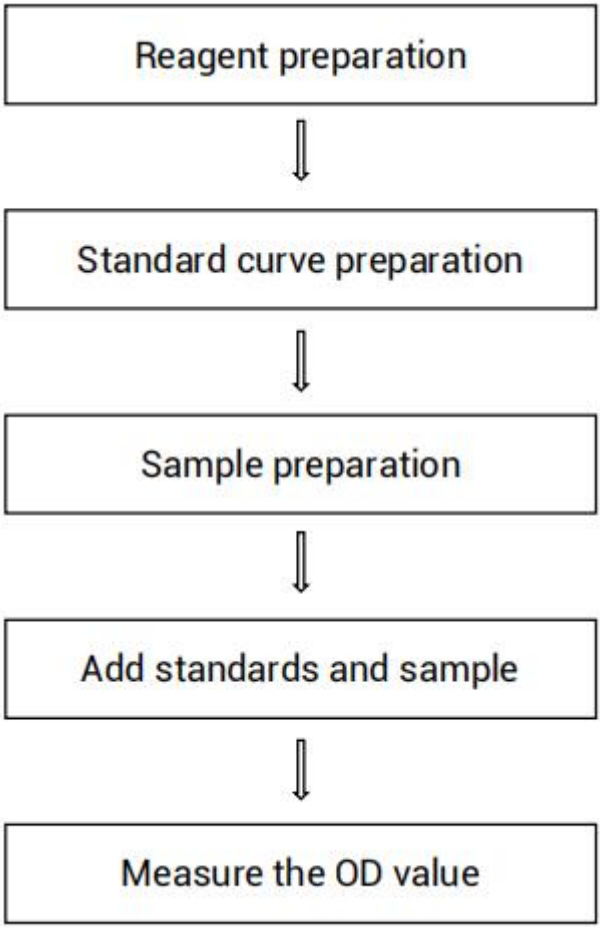
Assay summary

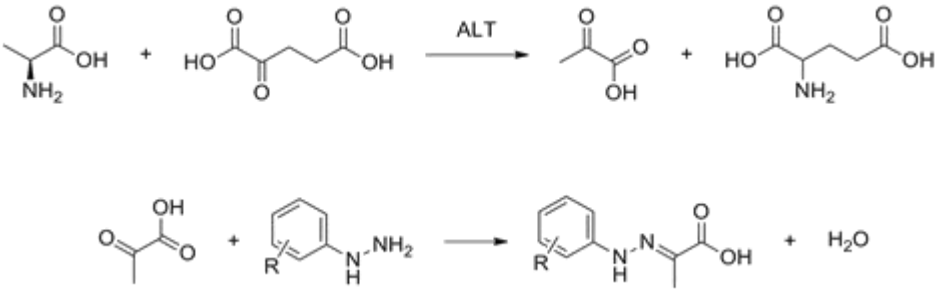


Intended use

This kit can be used to measure ALT/GPT activity in animal serum (plasma), tissue, culture cells and cell culture supernatant, etc.

Detection principle

Alanine aminotransferase (ALT) catalyze the amino conversion reaction between alanine and α-ketoglutaric acid to produce pyruvic acid and glutamic acid at pH 7.4 and 37°C. Then phenylhydrazine was added to form phenylhydrazone with pyruvic acid. Phenylhydrazone is reddish brown under alkaline conditions. ALT activity can be calculated by measuring the OD values at 510 nm.



Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 1	Buffer Solution	0.5 mL×1 vial	0.5 mL×1 vial	2.5 mL×1 vial	2-8°C, 12 months
Reagent 2	2 mmol/L Sodium Pyruvate	0.5 mL×1 vial	0.5 mL×1 vial	2.5 mL×1 vial	2-8°C, 12 months
Reagent 3	Substrate Solution	2.5 mL×1 vial	5 mL×1 vial	25 mL×1 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent	2.5 mL×1 vial	5 mL×1 vial	25 mL×1 vial	2-8°C, 12 months shading light
Reagent 5	Alkali Reagent	2.5 mL×1 vial	5 mL×1 vial	25 mL×1 vial	2-8°C, 12 months
	Microplate	48 wells	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 (500-520 nm), Micropipettor, Vortex mixer, Incubator, Multichannel pipette

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① The preparation of alkali working solution：
For each well, prepare 200 μL of alkali working solution (mix well 20 μL of alkali reagent and 180 μL of double distilled water). The alkali working solution should be prepared on spot.

② Incubate substrate solution at 37°C for 10 min.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Urine: collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant and preserve it on ice for detection.

Tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

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

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300 μL PBS (0.01M, pH 7.4) with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ 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
Human serum	1
Human plasma	1
Porcine serum	1
Rat serum	1
10% Mouse brain tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Mouse liver tissue homogenization	40-60
10% Mouse kidney tissue homogenization	1

Note: The diluent is saline (0.9% NaCl) or 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 It is recommended to use multi-channel pipette to add alkali working solution to reduce the difference between wells.

② The sample should be tested as soon as possible after collection. If testing cannot be performed immediately, it can be stored at -20°C for up to 20 days. Avoid repeated freeze-thaw cycles.

Operating steps

① Standard wells: Add 5 μL of buffer solution to the standard wells respectively (multi-channel pipette is recommended to be used). Add 20, 18, 16, 14, 12, 10 μL of substrate solution to the standard wells from A to F, respectively. Add 0, 2, 4, 6, 8, 10 μL of 2 mmol/L sodium pyruvate to the standard wells from A to F, respectively.

Sample wells: Add 20 μL of substrate solution (pre-heated at 37°C for 10 min) and 5 μL of sample.

Control wells: Add 20 μL of substrate solution (pre-heated at 37°C for 10 min).

② Mix well (this is very important), then incubate at 37°C for 30 min.

③ Add 20 μL of chromogenic agent to each well.

④ Control wells: Add 5 μL of sample to control wells.

⑤ Mix well with microplate reader for 10 s, incubate at 37°C for 20 min.

⑥ Add 200 μL of alkali working solution to each well (the multi-channel pipette is recommended).

⑦ Mix fully with microplate reader for 10 s, stand for 15 min at room temperature and measure the OD value of each well with microplate reader at 510 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #A) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration (0, 28, 57, 97, 150, 200) as x-axis and y-axis respectively. Create the standard curve (**$y = ax^2+bx + c$**) with graph software (or EXCEL).

Definition of international unit: The enzyme amount of 1 μmol of NADH consumed in reaction system (1 mL sample or 1 g tissue protein, 25°C) per minute is defined as 1 unit (wavelength is 340 nm, optical path is 1 cm).

Definition of Carmen unit: 1 mL of sample, the total volume of reaction is 3 mL, wavelength is 340 nm, optical path is 1 cm, react at 25°C for 1 min, the amount of generated pyruvic acid which oxidize NADH to NAD^+ and cause absorbance decreasing 0.001 is as 1 unit. (1 Carmen unit = 0.482 IU/L, 25°C).

The sample:

1. Serum (plasma) sample:

$$\text{ALT/GPT activity (IU/L)} = [a \times (\Delta A_{510})^2 + b \times \Delta A_{510} + c] \times 0.482 \times f$$

2. Tissue and cells sample:

$$\text{ALT/GPT activity (IU/gprot)} = [a \times (\Delta A_{510})^2 + b \times \Delta A_{510} + c] \times 0.482 \times f \div C_{pr}$$

[Note]

ΔA_{510}: OD_{sample}-$OD_{control}$

f: Dilution factor of sample before tested.

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

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 (IU/L)	3.50	26.50	52.60
%CV	5.7	5.2	5.0

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (IU/L）	3.50	26.50	52.60
%CV	8.4	9.2	9.4

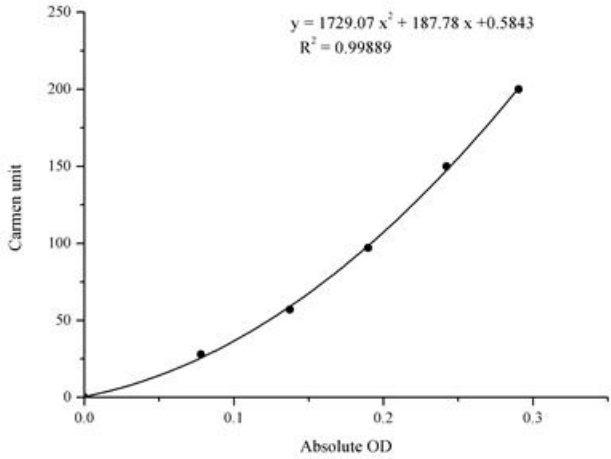
Sensitivity

The analytical sensitivity of the assay is 0.75 IU/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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Carmen unit	0	28	57	97	150	200
Average OD	0.247	0.324	0.384	0.436	0.489	0.537
Absoluted OD	0	0.078	0.137	0.190	0.242	0.290



Appendix II Example Analysis

Example analysis：

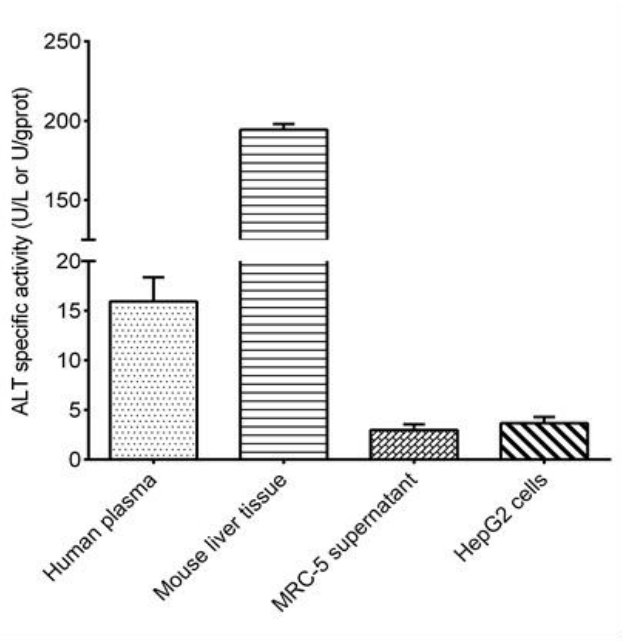
Take 5 μL of rabbit serum, carry the assay according to the operation steps. The results are as follows:

standard curve: $y = 1729.07 x^2 + 187.78 x + 0.5843$, the average OD value of the sample well is 0.303, the average OD value of the control well is 0.254, and the calculation result is:

$$\text{ALT activity (IU/L)} = [1729.07 \times (0.303 - 0.254)^2 + 187.78 \times (0.303 - 0.254) + 0.5843] \times 0.482$$

$$= 6.72 \text{ IU/L}$$

Detect human serum (V = 5 μL), 10% mouse liver tissue homogenate (the concentration of protein is 11.50 gprot/L, dilute for 50 times, V = 5 μL), culture supernatant of MRC-5 cells (V = 5 μL), HepG2 cells homogenate (the concentration of protein is 3.39 gprot/L, V = 5 μL) according to the protocol, the result is as follows：



Appendix III Publications

1. Fang X, Ding H, Chen Y, et al. Wireless Optogenetic Targeting Nociceptors Helps Host Cells Win the Competitive Colonization in Implant - Associated Infections[J]. Small Methods, 2024, 8(12): 2400216.

2. Lyu Y, Yang X, Yang L, et al. Lipid nanoparticle-mediated hepatocyte delivery of siRNA and silibinin in metabolic dysfunction-associated steatotic liver disease[J]. Journal of Controlled Release, 2024, 373: 385-398.

3. Li C, Wu Y, Chen K, et al. Gp78 deficiency in hepatocytes alleviates hepatic ischemia-reperfusion injury via suppressing ACSL4-mediated ferroptosis[J]. Cell Death & Disease, 2023, 14(12): 810.

4. Liu D, Zhan J, Wang S, et al. Chrysanthemum morifolium attenuates metabolic and alcohol-associated liver disease via gut microbiota and PPARα/γ activation[J]. Phytomedicine, 2024, 130: 155774.

5. Ma Y, Zhou W, Wang H, et al. The double-layer emulsions loaded with bitter melon (Momordica charantia L.) seed oil protect against dextran sulfate sodium-induced ulcerative colitis in mice[J]. International Journal of Biological Macromolecules, 2024, 278: 134279.

6. Wang Y, Wang J, Zhou T, et al. Investigating the potential mechanism and therapeutic effects of SLXG for cholesterol gallstone treatment[J]. Phytomedicine, 2024, 132: 155886.

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.