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

Catalog No：E-BC-F038

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.01-0.83 U/L

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

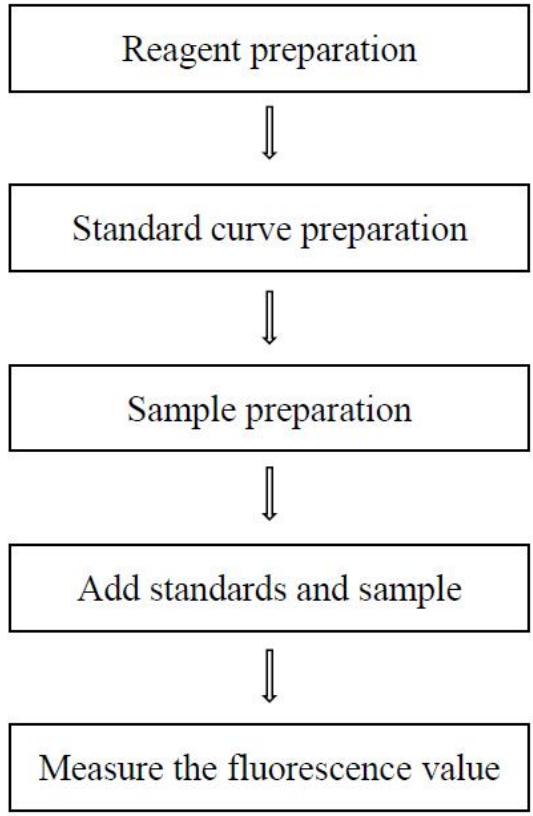
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 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.

Table of contents

Assay summary



Intended use

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

Detection principle

ALT catalyze the amino conversion reaction between alanine and α-ketoglutaric acid to produce pyruvic acid and glutamic acid. Under the action of pyruvate oxidase, pyruvic acid generates H_2O_2, which reacts with the non-fluorescent substance to form fluorescent substance under the action of peroxidase. The activity of ALT can be calculated by measuring the increase of fluorescence value at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	60 mL ×1 vial	60 mL × 2 vials	-20℃, 12 months
Reagent 2	Probe Solution	0.25 mL × 1 vial	0.5 mL × 1 vial	-20℃, 12 months shading light
Reagent 3	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months
Reagent 4	Substrate Solution	1.2 mL × 1 vial	1.2 mL × 2 vials	-20℃, 12 months shading light
Reagent 5	100 mmol/L Pyruvate Standard	0.1 mL × 1 vial	0.1 mL × 1 vial	-20℃, 12 months
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Pipettor, Vortex mixer, Centrifuge

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 1.2 mL of buffer solution and keep it on ice for use. Store at -20°C for 1 week.

③ The preparation of 1 mmol/L pyruvic acid standard stock solution:
Dilute 10 μL of 100 mmol/L pyruvate standard with 990 μL of buffer solution, mix well. The 1 mmol/L pyruvic acid standard stock solution should be prepared on spot.

④ The preparation of 50 μmol/L pyruvic acid standard solution:
Dilute 50 μL of 1 mmol/L pyruvic acid standard stock solution with 950 μL of buffer solution, mix well. The 50 μmol/L pyruvic acid standard solution should be prepared on spot and keep it on ice during use.

⑤ The preparation of reaction working solution:
For each well, prepare 100 uL of reaction working solution (mix well 56 μL of buffer solution, 4 μL of probe solution, 20 μL of enzyme working solution and 20 μL of substrate solution). The reaction working solution should be prepared on spot and protected from light.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 50 μmo/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 25, 30, 35, 40, 50 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**25**	**30**	**35**	**40**	**50**
50 μmol/L standard (μL)	0	40	80	100	120	140	160	200
Buffer solution (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Serum, plasma and other liquid sample: If the liquid sample is cloudy, centrifuge at 10000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection. If not detected on the same day, the serum, plasma or other liquid sample can be stored at -80℃ for a month

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 buffer solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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	10-15
Dog serum	5-10
Rat serum	10-15
10% Mouse heart tissue homogenate	100-120
10% Rat spleen tissue homogenate	10-15
10% Rat liver tissue homogenate	300-500
10% Rat kidney tissue homogenate	100-120
10% Rat lung tissue homogenate	100-120

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the corresponding well.
Sample well: add 20 μL of sample into the corresponding well.

② Add 100 μL of reaction working solution to each well.

③ Mix fully with microplate reader for 5 s and stand at room temperature for 3 min.

④ Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, recorded as F_1, and then react at room temperature for 60 min protected from light. The fluorescence intensity of each well was determined under the same wavelength, and recorded as F_2, then $\Delta F = F_2 - F_1$ (Note: There is no change in fluorescence value of standard well, plot the standard curve with the fluorescence value of F_2 (standard)).

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value ($F_{2(standard)}$) of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value ($\Delta F_{2(standard)}$) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) and other liquid sample:

Definition: The amount of enzyme in 1 L of serum or plasma that catalyze the production of 1 μmol pyruvic acid per minute at 25℃ is defined as 1 unit.

$$\text{ALT activity (U/L)} = (\Delta F - b) \div a \div T \times f$$

2. Tissue sample:

Definition: The amount of enzyme in 1 g of tissue protein that catalyze the production of 1 μmol pyruvic acid per minute at 25℃ is defined as 1 unit.

$$\text{ALT activity (U/gprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

[Note]

ΔF: The absolute fluorescence value of sample, $\triangle F = F_2 - F_1$.

T: The reaction time, 60 min.

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 (U/L)	0.10	0.35	0.65
%CV	2.5	2.3	2.1

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)	0.10	0.35	0.65
%CV	9.9	10.0	9.5

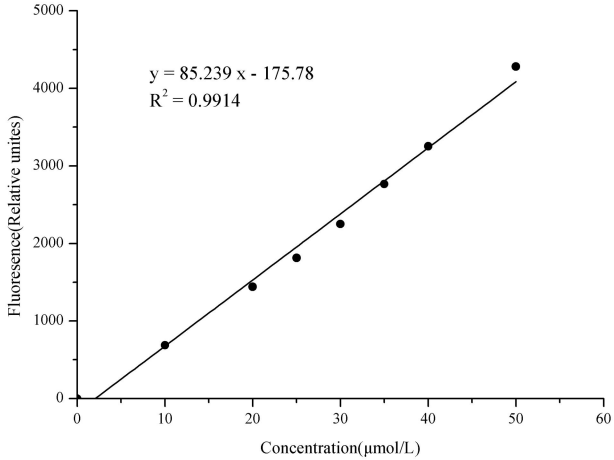
Sensitivity

The analytical sensitivity of the assay is 0.01 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.

2. Standard curve:

As the fluorescence 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:

Concentration (μmol/L)	**0**	**10**	**20**	**25**	**30**	**35**	**40**	**50**
Fluorescence value	1087	1806	2567	2922	3379	3906	4336	5384
	1150	1804	2550	2942	3362	3862	4408	5415
Average fluorescence value	1118	1805	2559	2932	3370	3884	4372	5399
Absoluted fluorescence value	0	687	1440	1814	2252	2766	3254	4281



Appendix II Example Analysis

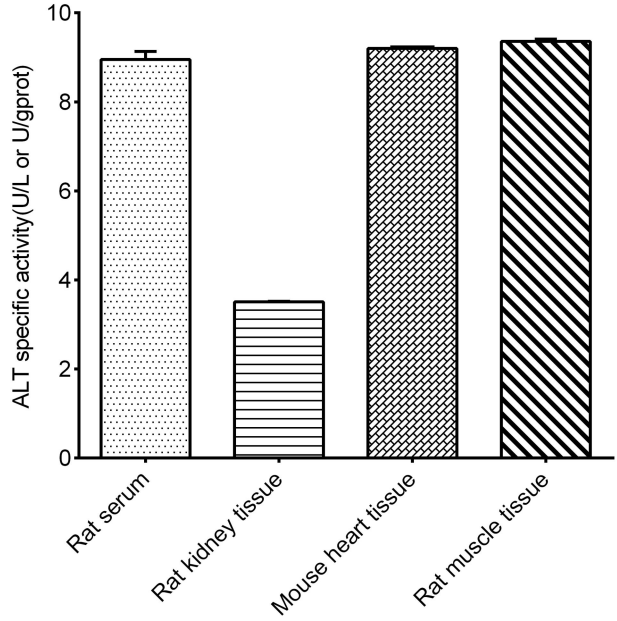
Example analysis:

For rat lung tissue, dilute supernatant of rat lung tissue homogenate for 100 times, take 20 μL of it to corresponding sample wells, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 69.312 x – 148.74, the average F_1 value of the sample is 611, the average F_2 value of the sample is 1577, $\Delta F = F_2-F_1$ =966, the concentration of protein in sample is 2.93 gprot/L, and the calculation result is:

ALT activity (U/gprot) = (966 + 148.74) ÷ 69.312 ÷ 60 × 100 ÷2.93 = 9.15 U/gprot

Detect rat serum (dilute for 10 times), 10% rat kidney tissue homogenate (the concentration of protein is 9.46 gprot/L dilute for 100 times), 10% mouse heart tissue homogenate (the concentration of protein is 3.24 gprot/L dilute for 100 times) and 10% rat muscle tissue homogenate (the concentration of protein is 3.62 gprot/L dilute for 100 times) 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.