Technical Manual

Alanine Aminotransferase (ALT/GPT) Activity Fluorometric Assay Kit

- Catalogue Code: MAES0009
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.01-0.83 U/L

Sensitivity:

0.01 U/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Alanine aminotransferase (ALT) is widely found in plasma and various tissues of the body, including liver, kidney, heart and skeletal muscle. ALT is an important pyridoxal phosphate dependent enzyme in the intermediate metabolism of glucose and protein. Clinically, the activity of serum alanine aminotransferase is often used as a marker for alcoholic liver disease, liver cirrhosis and acute viral hepatitis.

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	60 mL × 2 vials	-20°C, 6 months
Probe Solution	0.5 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Enzyme Reagent	Lyophilized × 2 vials	-20°C, 6 months
Substrate Solution	1.2 mL × 2 vials	-20°C, 6 months, avoid direct sunlight
Pyruvate Standard (100 mmol/L)	0.1 mL × 1 vial	-20°C, 6 months
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescence microplate reader (Ex/Em=535 nm/587 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

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

7. Reagent Preparation

1. Bring all reagent to room temperature before use.

2. Preparation of **enzyme working solution:** Dissolve a vial of enzyme reagent with 1.2 mL of buffer solution fully and preserve it on ice for use. The prepared enzyme working solution can be stored at -20°C for 1 week.

3. Preparation of **pyruvic acid standard stock solution (1 mmol/L):** Take 10 μL of reagent 5 to 990 μL of buffer solution and mix fully. Prepare the fresh solution before use.

4. Preparation of **pyruvic acid standard (50 μmol/L):** Dilute pyruvic acid standard stock solution (1 mmol/L) with buffer solution at the ratio of 1: 19 and mix fully. Prepare the fresh solution before use and preserve it on ice for use.

5. Preparation of **reaction working solution:** Mix the buffer solution, probe solution, enzyme working solution and substrate solution at a ratio of 56: 4: 20: 20. Prepare the fresh solution before use and stored with avoid direct sunlight.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of buffer solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.01-0.83 U/L).

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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em=535 nm/587 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

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

The measurement of samples

1. **Standard well:** Add 20 μL of standard with different concentrations into the well.
   Sample well: Add 20 μL of sample into the wells.

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 with avoid direct sunlight. 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)).

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Operation Table

	Standard well	Sample well
Sample (μL)		20
Standard with different concentrations (μL)	20	
Reaction working solution (μL)	100	100
Mix fully with microplate reader for 5 s and stand at room temperature for 3 min. 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 with avoid direct sunlight. The fluorescence intensity of each well was determined under the 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 $F_{2(standard)}$).		

11. Calculations

Plot the standard curve by using fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value of sample. The standard curve is: y= ax + b.

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.

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

y: The absolute fluorescence value of standard, $F_{Standard} - F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔF: Absolute fluorescence intensity of sample ($\triangle F = F_2 - F_1$)

T: The reaction time, 60 min

C_{pr}: The concentration of protein in sample, gprot/L

f: Dilution factor of sample before tested

12. Performance Characteristics

Detection Range	0.01-0.83 U/L
Sensitivity	0.01 U/L
Average inter-assay CV (%)	9.8
Average intra-assay CV (%)	2.3

Analysis

For rat lung tissue, dilute supernatant of rat lung tissue homogenate for 100 times, take 20 μL of it to corresponding sample wells, carry the assay according to the operation table.

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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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