TECHNICAL MANUAL

Alanine Aminotransferase (ALT/GPT) Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0009
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure fluorescence

2. Intended use

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

3. Detection principle

ALT catalyzes 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.

4. 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 °C, 12 months
Reagent 2	Probe Solution	0.25 mL × 1 vial	0.5 mL × 1 vial	-20 °C, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20 °C, 12 months
Reagent 4	Substrate Solution	1.2 mL × 1 vial	1.2 mL × 2 vials	-20 °C, 12 months, shading light
Reagent 5	100 mmol/L Pyruvate Standard	0.1 mL × 1 vial	0.1 mL × 1 vial	-20 °C, 12 months
	Black Microplate	96 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

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

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 small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **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. **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 fresh.
4. **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 fresh and kept on ice during use.
5. **Preparation of reaction working solution:** For each well, prepare 100 µL 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 fresh and protected from light.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 50 µmol/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.

7. Standard curve preparation

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

8. Sample preparation

Sample preparation:

Serum, plasma and other liquid samples: If the liquid sample is cloudy, centrifuge at 10,000 xg for 10 min at 4 °C. 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 °C 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 10,000 xg for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

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 perform a pretest to confirm the dilution factor.

9. The key points of the assay

Prevent the formation of bubbles when transferring supernatant into the microplate.

10. 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 incubate 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, 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 ΔF = 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. 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 absolute fluorescence value.

3. Plot the standard curve by using absolute fluorescence value (ΔF_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 catalyzes the production of 1 μmol pyruvic acid per minute at 25 °C is defined as 1 unit.

ALT activity (U/L) = (ΔF − b) ÷ a ÷ T × f

2. Tissue sample:

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

ALT activity (U/gprot) = (ΔF − b) ÷ a ÷ T × f ÷ C_{pr}

[Note]

ΔF: The absolute fluorescence value of sample, ΔF = 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.

12. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

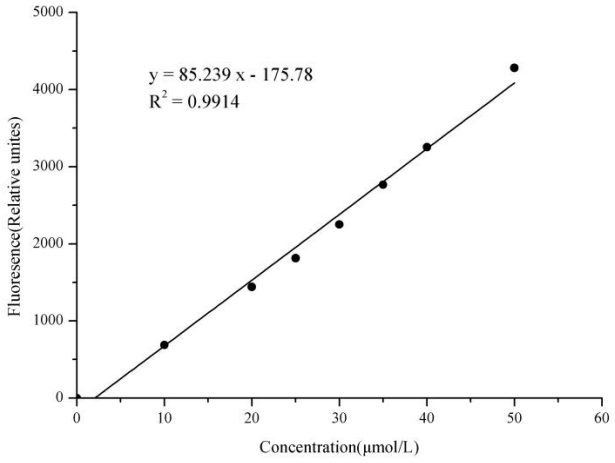
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.10	0.35	0.65
%CV	9.9	10.0	9.5

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.

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), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
0	1087	1150	1118	0
10	1806	1804	1805	687
20	2567	2550	2559	1440
25	2922	2942	2932	1814
30	3379	3362	3370	2252
35	3906	3862	3884	2766

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
40	4336	4408	4372	3254
50	5384	5415	5399	4281

13. Appendix II Example Analysis

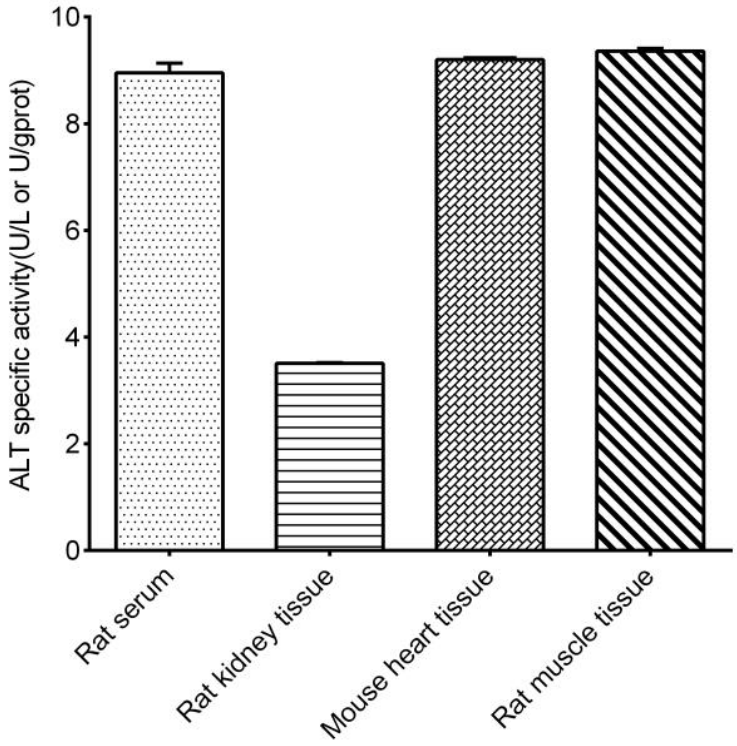
Example analysis:

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

Standard curve: y = 69.312x - 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 (diluted 10 times), 10% rat kidney tissue homogenate (the concentration of protein is 9.46 gprot/L diluted 100 times), 10% mouse heart tissue homogenate (the concentration of protein is 3.24 gprot/L diluted 100 times) and 10% rat muscle tissue homogenate (the concentration of protein is 3.62 gprot/L diluted 100 times) according to the protocol, the results are as follows:



14. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.