TECHNICAL MANUAL

Alanine Aminotransferase (ALT/GPT) Activity Assay Kit (Reitman-Frankel)

- **SKU CODE:** MAES0156
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

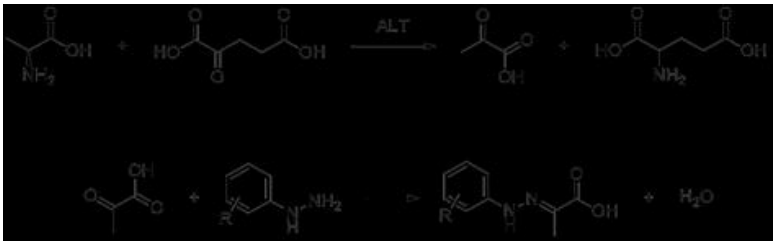
- Reagent preparation
- Sample preparation
- Standard curve preparation
- Add standards and samples
- Incubation and chromogenic reaction
- Measure absorbance at 510 nm

2. Intended use

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

3. Detection principle

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



4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	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

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
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, protected from 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	2 pieces		

5. 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)

6. Reagent preparation

1. **Preparation of alkali working solution:** For each well, prepare 200 µL of alkali working solution by mixing 20 µL of alkali reagent with 180 µL of double distilled water. The alkali working solution should be prepared fresh.
2. Incubate substrate solution at 37°C for 10 min before use.

7. Sample preparation

1. **Sample preparation:** Serum and plasma: Detect directly. If not tested on the same day, serum or plasma can be stored at -80°C for one month. Urine: Collect fresh

urine and centrifuge at 10,000×g for 15 min at 4°C. Take the supernatant and preserve on ice for detection. Tissue sample: ① Harvest the required amount of tissue 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 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep on ice for detection. ⑤ Meanwhile, determine the protein concentration of supernatant (MAES0177). Cells: ① Harvest the required number of cells 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 (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4°C. ④ Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep on ice for detection. ⑤ Meanwhile, determine the protein concentration of supernatant (MAES0177).

2. **Sample dilution:** The recommended dilution factors for different samples are as follows (for reference only): Human serum: 1 Human plasma: 1 Porcine serum: 1 Rat serum: 1 10% Mouse brain tissue homogenate: 1 10% Mouse heart tissue homogenate: 1 10% Mouse liver tissue homogenate: 40-60 10% Mouse kidney tissue homogenate: 1 Note: The diluent is saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For other sample types, perform a pre-test to confirm the dilution factor.

8. The key points of the assay

1. It is recommended to use a multichannel pipette to add alkali working solution to reduce variation between wells.

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

9. Operating steps

1. Standard wells: Add 5 µL of buffer solution to the standard wells (multichannel pipette recommended). Add 20, 18, 16, 14, 12, 10 µL of substrate solution to standard wells A through F, respectively. Add 0, 2, 4, 6, 8, 10 µL of 2 mmol/L sodium pyruvate to standard wells A through 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 (multichannel pipette recommended).
7. Mix thoroughly with microplate reader for 10 s, stand for 15 min at room temperature and measure the absorbance of each well at 510 nm.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using absolute absorbance values of standards and corresponding concentrations (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 that consumes 1 µmol of NADH in the reaction system (1 mL sample or 1 g tissue protein, 25°C) per minute is defined as 1 unit (wavelength 340 nm, optical path 1 cm).

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

The sample:

1. Serum (plasma) sample:

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 cell sample:

ALT/GPT activity (IU/g_{prot}) = $[a \times (\Delta A\_510)^2 + b \times \Delta A\_510 + c] \times 0.482 \times f \div C_{pr}$

[Note]

ΔA_510: OD_{sample} - $OD_{control}$

f: Dilution factor of sample before testing

C_{pr}: Concentration of protein in sample (g_{prot}/L)

11. 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 (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.

Inter-assay Precision

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 absorbance obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

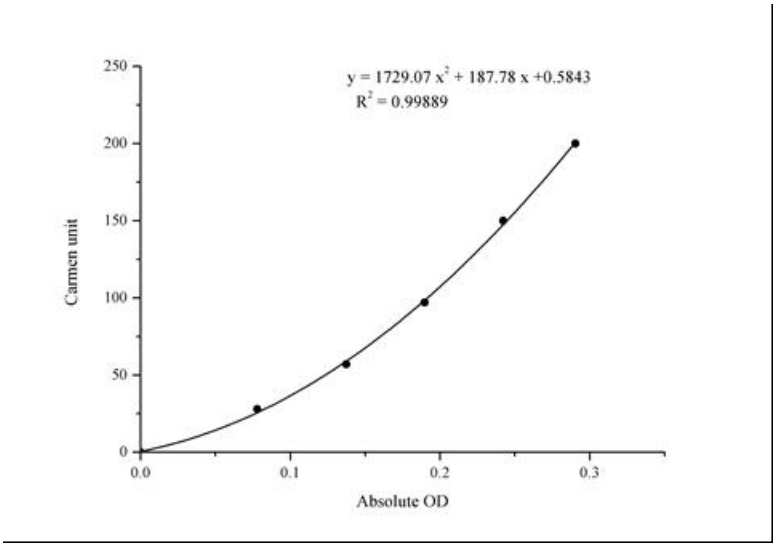
Standard curve

As the absorbance values 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

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

Standard curve



12. Appendix II Example Analysis

Example analysis:

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

Standard curve: $y = 1729.07x^2 + 187.78x + 0.5843$

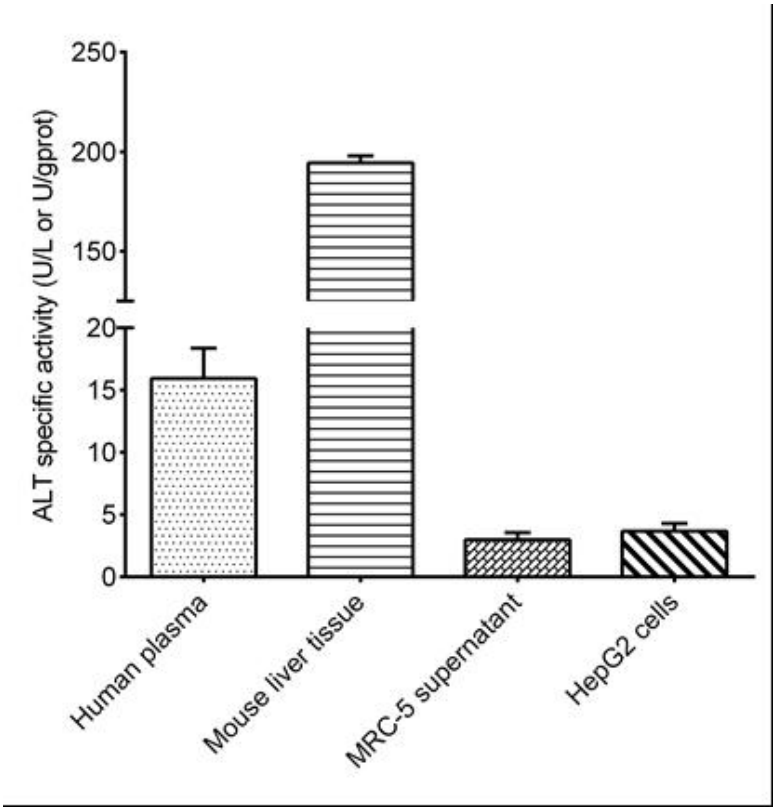
Average absorbance of sample well: 0.303

Average absorbance of control well: 0.254

Calculation result:

ALT activity (IU/L) = [1729.07 × (0.303 - 0.254)^2 + 187.78 × (0.303 - 0.254) + 0.5843] × 0.482 = 6.72 IU/L

Detect human serum (V = 5 µL), 10% mouse liver tissue homogenate (protein concentration 11.50 g_{prot}/L, diluted 50 times, V = 5 µL), culture supernatant of MRC-5 cells (V = 5 µL), HepG2 cell homogenate (protein concentration 3.39 g_{prot}/L, V = 5 µL) according to the protocol. The results are as follows:



13. 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.