Technical Manual

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

- Catalogue Code: MAES0157
- Size: 100 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

1.26-72.3 IU/L

Sensitivity:

1.26 IU/L

Storage:

2-8°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 transaminase (ALT) is a transaminase enzyme. It is also called alanine aminotransferase (ALAT) and was formerly called serum glutamate-pyruvate transaminase (SGPT) or serum glutamic-pyruvic transaminase (SGPT). ALT is found in plasma and in various body tissues, but is most common in the liver. It catalyzes the two parts of the alanine cycle. Serum ALT level, serum AST (aspartate transaminase) level, and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health.

3. Intended Use

This kit can be used to measure ALT/GPT activity in serum, plasma, tissue, culture supernatant and other samples.

4. Detection Principle

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.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	1.8 mL × 1 vial	2-8°C, 6 months
Sodium Pyruvate (2 mmol/L)	1.8 mL × 1 vial	2-8°C, 6 months
Substrate Solution	30 mL × 2 vials	2-8°C, 6 months
Chromogenic Agent	30 mL × 2 vials	2-8°C, 6 months, avoid direct sunlight
Alkali Reagent	30 mL × 2 vials	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (505 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

Detect the sample as soon as possible after collection. The serum sample can be store at 2-8°C for 7 days and -20°C for 20 days.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **alkali working solution**: Dilute the alkali reagent with double distilled water at the ratio of 1: 9 and mix fully. Prepare the fresh solution before use.

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

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. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): PBS (0.01 M, pH 7.4) including 0.1 mM EDTA (μL) =1: 400. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. 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 PBS (0.01 M, pH 7.4) including 0.1 mM EDTA (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 (1.26-72.3 IU/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
10% Rat liver tissue homogenization	30-60
10% Rat kidney tissue homogenization	1
Human serum	1
Human plasma	1
HepG2 cells homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 505 nm

10. Operation Steps

The preparation of standard curve

1. **Standard tubes:** Record the test tube with A, B, C, D, E, F in duplication, add 0.1 mL of buffer solution to the standard tubes respectively. Add 0, 0.05, 0.10, 0.15, 0.20, 0.25 mL of sodium pyruvate to the standard tubes from A to F, respectively. Add 0.50, 0.45, 0.40, 0.35, 0.30, 0.25 mL of substrate solution to the standard tubes from A to F, respectively.

2. Add 0.50 mL of chromogenic agent to each tube.

3. Mix fully and incubate at 37°C for 20 min.

4. Add 5 mL of alkali working solution to each tube.

5. Stand for 10 min at room temperature and set to zero with double distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm.

The measurement of samples

1. **Sample tubes:** Add 0.5 mL of substrate solution (pre-heated at 37°C for 10 min) and 0.1 mL of sample.
   Control tubes: Add 0.5mL of substrate solution (pre-heated at 37°C for 10 min).

2. Mix fully and incubate at 37°C for 30 min.

3. Add 0.50 mL of chromogenic agent to each tube.

4. **Control tubes:** Add 0.1 mL of sample to Control tubes.

5. Mix fully and incubate at 37°C for 20 min.

6. Add 5 mL of alkali working solution to each tube.

7. Stand for 10 min at room temperature and set to zero with double distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm

Operation Table

The preparation of standard curve

Set 6 tubes of micro-plate for standard and operate according to the following operating table.

	A	B	C	D	E	F
Buffer solution (mL)	0.10	0.10	0.10	0.10	0.10	0.10
Sodium pyruvate (2 mmol/L) (mL)	0	0.05	0.10	0.15	0.20	0.25
Substrate solution (mL)	0.50	0.45	0.40	0.35	0.30	0.25
Chromogenic agent (mL)	0.50	0.50	0.50	0.50	0.50	0.50
Mix fully (this is very important), then incubate at 37°C for 30 min						
Alkali working solution (mL)	5	5	5	5	5	5
Stand for 10 min at room temperature and set to zero with double distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm						

The measurement of samples

	Control tube	Sample tube
Sample (mL)		0.1
Substrate solution (mL) (pre-heated at 37°C for 10 min)	0.5	0.5
Mix fully (this is very important), then incubate at 37°C for 30 min.		
Chromogenic agent (mL)	0.5	0.5
Sample (mL)	0.1	
Mix fully and incubate at 37°C for 20 min.		
Alkali working solution (mL)	5	5
Stand for 10 min at room temperature and set to zero with double distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm		

11. Calculations

1. **Definition of international unit:** The enzyme amount of 1 μmol of NADH consumed in reaction system per minute at 25°C is defined as 1 unit.

2. **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**).

3. Plot the standard curve by using OD value of standard and correspondent Carmen unit (0, 28, 57, 97, 150, 200 Carmen unit) as x-axis and y-axis respectively. Create the standard curve with graph software (or EXCEL). The Carmen unit of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is $y=ax^2+bx+c$.

1. Serum (plasma) sample:

$$\underset{(IU/L)}{\text{ALT activity}} = [a \times (\Delta A_{505})^2 + b \times \Delta A_{510} + c] \times 0.482\ IU/L \times f$$

2. Tissue and Cells sample:

$$\underset{(IU/L)}{\text{ALT activity}} = [a \times (\Delta A_{505})^2 + b \times \Delta A_{510} + c] \times 0.482\ IU/L \times f \div C_{pr}$$

y: Carmen unit.
x: $OD_{Standard} - OD_{Blank}$ （ OD_{Blank} is the OD value when the carmen unit is 0）
a, b, c: the constant of standard curve.
ΔA_{505}: OD_{sample}-$OD_{control}$
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

12. Performance Characteristics

Detection Range	1.26-72.3 IU/L
Sensitivity	1.26 IU/L
Average inter-assay CV (%)	8.2
Average intra-assay CV (%)	4.3

Analysis

Take 0.1 mL of human serum, carry the assay according to the operation table.

The results are as follows:

standard curve: $y = 629.84\ x^2 + 115.13\ x + 2.0232$, the average OD value of the sample is 0.288, the average OD value of the control is 0.240, and the calculation result is:

$$\text{ALT activity (IU/L)} = [629.84 \times (0.288 - 0.240)^2 + 115.13 \times (0.288 - 0.240) + 2.0232] \times 0.482$$

$$= 4.34\ \text{IU/L}$$

Email: info@assaygenie.com **Web:** www.assaygenie.com

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent 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.

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

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.

Contact Details

Email: info@assaygenie.com

Web: www.assaygenie.com