# Technical Manual

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

- Catalogue Code: MAES0156
- Size: 96T
- Research Use Only

# 1. Key Features and Sample Types:

## Detection method:

Colorimetric method

## Specification:

96T

## Range:

0.75-72.3 IU/L

## Sensitivity:

0.75 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 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 ALT/GPT activity in animal serum (plasma), tissue and culture cells etc.

## 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** | 0.5 mL × 1 vial | 2-8°C, 6 months |
| **Sodium Pyruvate (2 mmol/L)** | 0.5 mL × 1 vial | 2-8°C, 6 months |
| **Substrate Solution** | 5 mL × 1 vial | 2-8°C, 6 months, avoid direct sunlight |
| **Chromogenic Agent** | 5 mL × 1 vial | 2-8°C, 6 months, avoid direct sunlight |
| **Alkali Reagent** | 5 mL × 1 vial | 2-8°C, 6 months |
| **Microplate** | 96 wells | No requirement |
| **Plate Sealer** | 2 pieces | |

### Materials required but not supplied

- Micropipettor
- Vortex mixer
- Incubator
- Multichannel pipette
- Microplate Reader (500-520 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:

1. It is recommended to use a multi-channel pipette to add alkali working solution to reduce the difference between wells.

2. Detect the sample as soon as possible after collection. The serum sample can be stored 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 ($2\times10^6$): 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 (0.75-72.3 IU/L).

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

| Sample Type: | Dilution Factor: |
|---|---|
| Human serum | 1 |
| Human plasma | 1 |
| Porcine serum | 1 |
| Rat serum | 1 |
| 10% Rat brain tissue homogenization | 1 |
| 10% Rat heart tissue homogenization | 1 |
| 10% Rat liver tissue homogenization | 40-60 |
| 10% Rat kidney tissue 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:** 510 nm

### Plate Set Up:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | A | A | S3' | S3 | S11' | S11 | S19' | S19 | S27' | S27 | S35' | S35 |
| **B** | B | B | S4' | S4 | S12' | S12 | S20' | S20 | S28' | S28 | S36' | S36 |
| **C** | C | C | S5' | S5 | S13' | S13 | S21' | S21 | S29' | S29 | S37' | S37 |
| **D** | D | D | S6' | S6 | S14' | S14 | S22' | S22 | S30' | S30 | S38' | S38 |
| **E** | E | E | S7' | S7 | S15' | S15 | S23' | S23 | S31' | S31 | S39' | S39 |
| **F** | F | F | S8' | S8 | S16' | S16 | S24' | S24 | S32' | S32 | S40' | S40 |
| **G** | S1' | S1 | S9' | S9 | S17' | S17 | S25' | S25 | S33' | S33 | S41' | S41 |
| **H** | S2' | S2 | S10' | S10 | S18' | S18 | S26' | S26 | S34' | S34 | S42' | S42 |

**Note:** A-F, standard wells; S1'-S42', control wells; S1-S42, sample wells.

## 10. Operation Steps:

1. **Standard wells:** Add 5 µL of buffer solution to the standard wells respectively (multi-channel pipette is recommended to be used). Add 20, 18, 16, 14, 12, 10 µL of substrate solution to the standard wells from A to F, respectively. Add 0, 2, 4, 6, 8, 10 µL of 2 mmol/L sodium pyruvate to the standard wells from A to 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 fully (this is very important), then incubate at 37°C for 30 min.

3. Add 20 µL of chromogenic agent to each well

4. Add 5 µL of sample to **control wells**.

5. Mix fully with microplate reader for 10 s, incubate at 37°C for 20 min.

6. Add 200 µL of alkali working solution to each well (the multi-channel pipette is recommended).

7. Mix fully with microplate reader for 10 s, stand for 15 min at room temperature and measure the OD value of each well with microplate reader at 510 nm.

## Operation Table

### The preparation of standard curve

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

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| **Buffer solution (μL)** | 5 | 5 | 5 | 5 | 5 | 5 |
| **Substrate solution (μL)** | 20 | 18 | 16 | 14 | 12 | 10 |
| **Sodium pyruvate (2 mmol/L) (μL)** | 0 | 2 | 4 | 6 | 8 | 10 |
| Mix fully (this is very important), then incubate at 37°C for 30 min | | | | | | |
| **Chromogenic agent (μL)** | 20 | 20 | 20 | 20 | 20 | 20 |
| Mix fully with microplate reader for 10s and incubate at 37°C for 20 min | | | | | | |
| **Alkali working solution (μL)** | 200 | 200 | 200 | 200 | 200 | 200 |
| Mix fully with microplate reader for 10s, stand for 15 min at room temperature and measure the OD value of each well with microplate reader at 510 nm | | | | | | |

### The measurement of samples

| | Control well | Sample well |
|---|---|---|
| **Substrate solution (μL)** (pre-heated at 37°C for 10 min) | 20 | 20 |
| **Sample (μL)** | | 5 |
| Mix fully (this is very important), then incubate at 37°C for 30 min | | |
| **Chromogenic agent (μL)** | 20 | 20 |
| **Sample (μL)** | 5 | |
| Mix fully with microplate reader for 10s and incubate at 37°C for 20 min | | |
| **Alkali working solution (μL)** | 200 | 200 |
| Mix fully with microplate reader for 10s, stand for 15 min at room temperature and measure the OD value of each well with microplate reader at 510 nm | | |

## 11. Calculations:

1. Definition of international unit: The enzyme amount of 1 μmol of NADH consumed in reaction system (1 mL sample or 1 g tissue protein, 25°C) per minute is defined as 1 unit (wavelength is 340 nm, optical path is 1 cm).

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:**

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

**2. Tissue and Cells sample:**

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

**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.
**$\Delta A_{510}$:** $OD_{sample}$-$OD_{control}$
**f:** Dilution factor of sample before test.
**$C_{pr}$:** Concentration of protein in sample, gprot/L.

## 12. Performance Characteristics:

| | |
|---|---|
| **Detection Range** | 0.75-72.3 IU/L |
| **Sensitivity** | 0.75 IU/L |
| **Average inter-assay CV (%)** | 9 |
| **Average intra-assay CV (%)** | 5.3 |

### Analysis

Take 5 μL of rabbit serum, carry the assay according to the operation table.

**The results are as follows:**

standard curve: $y = 1729.07 x^2 + 187.78 x + 0.5843$, the average OD value of the sample well is 0.303, the average OD value of the control well is 0.254, the calculation result is:

$$\text{ALT activity (IU/L)} =$$

$$[1729.07 \times (0.303 - 0.254)^2 + 187.78 \times (0.303 - 0.254) + 0.5843] \times 0.482$$

$$= 6.72 \text{ IU/L}$$



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## 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.



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## Notes:

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