Alanine Aminotransferase (ALT/GPT) Activity Assay Kit

Catalogue No.: K097

Size: 96T(42 samples)

Range: 0.75-72.30IU/L

Sensitivity: 0.75IU/L

Kit component:

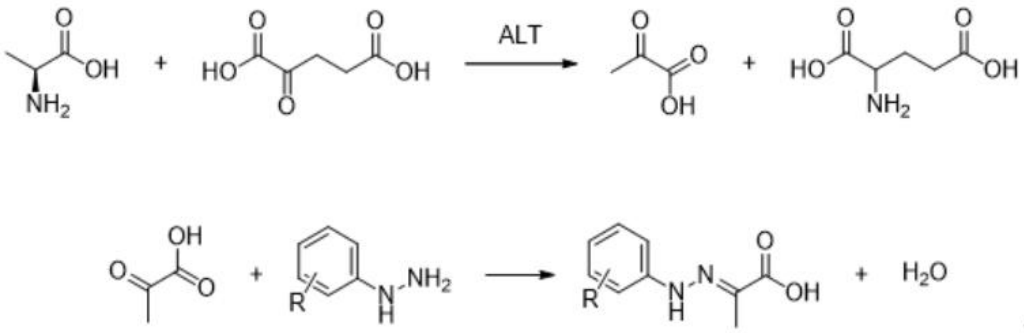
Item	Component	Size（96T）	Storage
Reagent 1	Buffer Solution	0.5 mL	2-8℃
Reagent 2	2 mmol/L Sodium Pyruvate	0.5 mL	2-8℃
Reagent 3	Substrate Solution	5 mL	2-8℃
Reagent 4	Chromogenic Agent	5 mL	2-8℃ shading light
Reagent 5	Alkali Reagent	5 mL	2-8℃
	Product Description	1 copy	

Storage:

The kit should be stored at 2-8℃ shading light for 6 months.

Principle of the Assay:

Alanine aminotransferase (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.



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

Assay Procedure (For reference):

1. **Reagent preparation**

a. Bring all reagents to room temperature before use.

b. The preparation of alkali working solution:

Mix alkali reagent and double distilled water thoroughly at the volume ratio of 1:9. The alkali working solution should be prepared on spot.

c. Take part of substrate solution, and incubate it at 37°C for 10 min.

2. Sample preparation

a. **Preparation of sample**

Serum and plasma: detect directly.

Urine: collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant and preserve it on ice for detection.

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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (K001).

Cells:

① Harvest the number of cells needed 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 (0.01M, pH 7.4) with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (K001).

b. **Dilution of sample**

Before the formal test, 2-3 samples with significant differences should be selected and diluted into different concentrations for pre-test. Determine whether dilution is required according to the pre-test results and the kit detection range of 0.75-72.30 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 saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

3. Add samples and test

① Standard wells: Add 5 μL of Reagent 1(buffer solution) to the standard wells respectively (multi-channel pipette is recommended to be used). Add 20, 18, 16, 14, 12, 10 μL of Reagent 3(substrate solution) to the standard wells from A to F, respectively. Add 0, 2, 4, 6, 8, 10 μL of Reagent 2(2 mmol/L sodium pyruvate) to the standard wells from A to F, respectively.

Sample wells: Add 20 μL of Reagent 3(substrate solution) (pre-heated at 37°C for 10 min) and 5 μL of sample.

Control wells: Add 20 μL of Reagent 3(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 Reagent 4(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 (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 Sheet

Standard Curve

	A	B	C	D	E	F
Reagent 1(μL)	5	5	5	5	5	5
Reagent 3(μL)	20	18	16	14	12	10
Reagent 2(μL)	0	2	4	6	8	10

After each addition of Reagent 2, mix by aspirating and dispensing the solution with the pipette tip submerged in the well liquid. After the addition is complete, incubate at 37°C for 30 minutes.						
Reagent 4(μL)	20	20	20	20	20	20
Mix well with microplate reader for 10 s, incubate at 37°C for 20 min.						
Alkali working solution(μL)	200	200	200	200	200	200
Mix on a microplate shaker for 10 seconds, then incubate at room temperature for 15 minutes. Measure the optical density (OD) of each well at 510 nm using a microplate reader. Plot a quadratic (second-order polynomial) curve, using the absolute OD values of the standard wells as the X-axis and the corresponding 0, 28, 57, 97, 150, 200 Kárman units as the Y-axis.						

Sample Assay

	Control wells	Sample wells
Reagent 3(μL) pre-heated at 37°C for 10 min	20	20
Samples(μL)	--	5
Prior to incubation, thoroughly mix each sample directly in the assay well by pipetting up and down after dispensing. Then, incubate at 37°C for 30 minutes.		
Reagent 4(μL)	20	20
Samples(μL)	5	--
After each addition of a sample to the control well, mix by pipetting up and down with the tip immersed in the well liquid. Following the final addition, mix the plate on a microplate shaker for 10 seconds and then incubate at 37°C for 20 minutes.		
alkali working solution(μL)	200	200
Mix the plate on a microplate shaker for 10 seconds and then incubate at room temperature for 15 minutes. Measure the optical density (OD) of each well at 510 nm using a microplate reader. The absolute OD value of the sample (calculated as the OD of the sample well minus the OD of the control well) is then substituted into the curve formula for calculation.		

Calculation:

the standard curve: $y = a x^2 + b x + c$

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)

Definition of international unit: One unit is defined as the amount of enzyme that catalyzes the reduction of 1 μmol of NADH per minute at 25°C.

For Serum (plasma) and Cell culture supernatant sample:

ALT/GPT activity (IU/L) = [a × (ΔA510) ^{2} + b × ΔA510+ c] × 0.482 IU/L* × f

For Tissue and cells sample:

ALT/GPT activity (IU/gprot) = [a × (ΔA510) ^{2} + b × ΔA510+ c] × 0.482 IU/L* × f ÷ Cpr

Note:

y: Kárman units (0, 28, 57, 97, 150, 200)

x: Standard OD value - Blank OD value (i.e., the OD value at 0 Kárman unit)

a, b, c: Constants corresponding to the fitted curve

ΔA510: OD_{sample}-$OD_{control}$

*: Under the condition of 25°C, one Kárman unit equals 0.482 IU/L.

f: Dilution factor of sample before tested.

Cpr: Concentration of protein in sample (gprot/L).

Notes:

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.

2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.

3. Protection methods must be taken by wearing a lab coat and latex gloves during the experiment.

4. The detection range of the kit is not equivalent to the concentration range of the substance to be measured in the sample. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.

5. If the samples to be tested are not among the types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.

6. The experimental results are closely related to the situation of reagents, operations, environment and so on. FineTest will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.