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

Catalog No: E-BC-K235-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 44 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 1.26 IU/L

Detection range: 1.26-72.3 IU/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

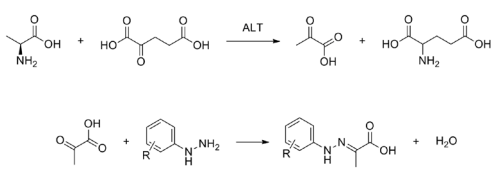
This kit can be used to measure ALT/GPT activity in serum, plasma, tissue, culture supernatant and other samples.

▲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.

▲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℃ . 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.



▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	1.8 mL × 1 vial	2-8℃ , 6 months
Reagent 2	2 mmol/L Sodium Pyruvate	1.8 mL × 1 vial	2-8℃ , 6 months
Reagent 3	Substrate Solution	30 mL × 2 vials	2-8℃ , 6 months
Reagent 4	Chromogenic Agent	30 mL × 2 vials	2-8℃ , 6 months, shading light
Reagent 5	Alkali Reagent	30 mL × 2 vials	2-8℃ , 6 months

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.

▲Materials prepared by users

Instruments

UV-visible Spectrophotometer Spectrophotometer (505 nm), Micropipettor, Vortex mixer, 37℃ Constant temperature incubator

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key point of the assay

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

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 5 working solution:

   Dilute the Reagent 5 with double distilled water at the ratio of 1: 9 and mix fully. Prepare the fresh solution before use.

2. Incubate reagent 3 at 37 °C for 10 min.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	505 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. 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 regent 1 to the standard tubes respectively. Add 0, 0.05, 0.10, 0.15, 0.20, 0.25 mL of reagent 2 to the standard tubes from A to F, respectively. Add 0.50, 0.45, 0.40, 0.35, 0.30, 0.25 mL of reagent 3 to the standard tubes from A to F, respectively.

   (2) Add 0.50 mL of reagent 4 to each well.

   (3) Mix fully and incubate at 37℃ for 20 min.

   (4) Add 5 mL of reagent 5 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.

2. The measurement of samples

   (1) Sample tubes: Add 0.5 mL of reagent 3 (pre-heated at 37℃ for 10 min) and 0.1 mL of sample.

   Control tubes: Add 0.5mL of reagent 3 (pre-heated at 37℃ for 10 min).

   (2) Mix fully and incubate at 37℃ for 30 min.

   (3) Add 0.50 mL of reagent 4 to each tube.

   (4) Control tubes: Add 0.1 mL of sample to Control tubes.

   (5) Mix fully and incubate at 37℃ for 20 min.

   (6) Add 5 mL of reagent 5 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

	A	B	C	D	E	F
Reagent 1 (mL)	0.10	0.10	0.10	0.10	0.10	0.10
Reagent 2 (mL)	0	0.05	0.10	0.15	0.20	0.25
Reagent 3 (mL)	0.50	0.45	0.40	0.35	0.30	0.25
Reagent 4 (mL)	0.50	0.50	0.50	0.50	0.50	0.50
Mix fully (this is very important), then incubate at 37℃ for 20 min.						
Reagent 5 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 well	Sample well
Sample (mL)		0.1
Reagent 3 (mL) (pre-heated at 37℃ for 10 min)	0.5	0.5
Mix fully (this is very important), then incubate at 37℃ for 30 min.		
Reagent 4 (mL)	0.5	0.5
Sample (mL)	0.1	
Mix fully with microplate reader for 10 s and incubate at 37℃ for 20 min.		
Reagent 5 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.		

▲Calculation

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℃) 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℃ 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 Karmen unit = 0.482 IU/L, 25℃).
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

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

(2) Tissue and Cells

$$\text{ALT/GPT activity (IU/gprot)}=[a\times(\Delta A_{505})^2+b\times\Delta A_{505}+c]\times 0.482\times f\div Cpr$$

Note:

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 tested.

Cpr: concentration of protein in sample (gprot/L)

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

Appendix I Performance characteristics			
Detection range	1.26-72.3 IU/L	Average intra-assay CV (%)	4.3
Sensitivity	1.26 IU/L	Average inter-assay CV (%)	8.2

▲Example 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:

$$\text{ALT activity(IU/L)} = [629.84 \times (0.288 - 0.240)^2 + 115.13 \times (0.288 - 0.240) + 2.0232] \times 0.482 = 4.34\ \text{IU/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant and it is 10-12.5 IU of Heparin into 1 mL blood), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection.

▲ Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant to preserve it on ice for detection.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

▲ Cells

Collect the cells and wash the cells with homogenization medium 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 (10^5): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) including 0.1 mM EDTA.
2. Homogenized method:

   (1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

   Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.