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Alanine Aminotransferase (ALT/GPT) Activity Assay Kit

Catalog No.: BC00057

Size: 100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email（Sale） order@enkilife.com

✉Email（Techsupport） techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
Detection Methods	Colorimetric
Sample type	Serum, plasma, tissue, cells, cell supernatant
Detection Type	Enzyme activity
Detection instrument and wavelength	Microplate reader (510 nm)

Product Introduction

Alanine aminotransferase, also known as alanine aminotransferase, is mainly distributed in animal livers, but also exists in plants, microorganisms and cultured cells, and is essential for amino acid metabolism. In the diagnosis of liver diseases, liver cell damage can cause alanine aminotransferase to be released from the cells into the blood, thereby causing an increase in serum alanine aminotransferase activity. Therefore, alanine aminotransferase is a sensitive and commonly used indicator for assessing liver function damage.

Detection Principle

Alanine aminotransferase (ALT) acts on substrates composed of alanine and α-ketoglutarate at 37°C and pH 7.4 to generate pyruvate and glutamate. After 30 min of reaction (fixed time), 2,4-dinitrophenylhydrazine (DNPH) hydrochloric acid solution is added to terminate the reaction. At the same time, DNPH adds to the carbonyl group in ketoacid to generate pyruvate phenylhydrazone. Phenylhydrazone is reddish brown under alkaline conditions. The absorbance is measured at 510 nm and the enzyme activity is calculated.

Product Composition

Serial Number	Product Name	Packing Specifications (100T)	Storage
Reagent 1	Substrate matrix liquid	5m L	2-8 ℃ after opening .
Reagent 2	Color developer	5m L	2-8 ℃ away from light after
Reagent 3	Alkaline solution	5m L	2-8 ℃ after opening .
Reagent 4	2 μmol /mL Sodium	0.5mL	2-8 ℃ after opening .
Reagent 5	Buffer	0.5mL	2-8 ℃ after opening .

Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage conditions

The unopened test kit can be stored at -20℃ for 12 months, and after opening, it can be stored at 2-8 ℃ for 6 months.

Preparation before the experiment

Sample processing

1. Liquid samples such as serum (plasma): direct measurement.
2. Tissue samples: Tissue samples were homogenized with physiological saline (0.9% NaCl). After centrifugation, the supernatant was taken for determination, and part of the supernatant was used for protein concentration determination.
3. Cell samples: Cell samples were mechanically homogenized or ultrasonically disrupted using physiological saline (0.9% NaCl). After centrifugation of the homogenate, the supernatant was taken for determination, and part of the supernatant sample was used for protein concentration determination.

· Preparation of the kit

1. Before testing, the reagents were equilibrated to room temperature.
2. Reagent 3 working solution: Dilute reagent 3 and double distilled water in a volume ratio of 1:9 and prepare before use.
3. Take a portion of reagent 1 and preheat it in a 37°C incubator for 10 min.

Operation process

1. The standard curve operation table is as follows:

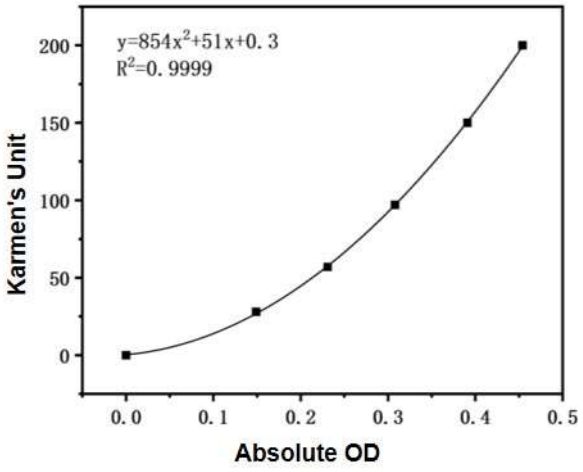
serial number	0	1	2	3	4	5
Reagent 5 (μL)	5	5	5	5	5	5
Reagent 4 (μL)	0	2	4	6	8	1 0
Reagent 1 (μL)	2 0	1 8	1 6	1 4	1 2	1 0
Reagent 2 (μL)	2 0	2 0	2 0	2 0	2 0	2 0
After mixing, react at 37 °C for 20 minutes						
Reagent 3 working	2 00	2 00	2 00	2 00	2 00	2 00
Gently shake the 96- well plate horizontally to mix, and place it at room temperature for 15 minutes. Use a microplate reader to measure the OD value of each well at a wavelength of 510						

nm . Subtract the absorbance of the zero well from the absorbance of each well. The difference = absolute OD value is used as the horizontal axis, and the corresponding Karman's unit is used as the vertical axis to make a coordinate graph fitting formula .

2. The measurement results table (with reference standard curve) is as follows:

OD value	0.41	0.559	0.641	0.718	0.801	0.864
Absolute OD value	0	0.149	0.231	0.308	0.391	0.454
Karman's unit	0	28	57	97	150	200

The standard curve needs to be made by the customer to be more accurate. The operation steps refer to the above operation table, and you don't need to draw the standard curve every time. The Karmen's unit value listed in the above table corresponds to the amount of standard sample added to each standard well, so the value is fixed. Customers can use this value and the absorbance value of each standard well obtained according to the operation table to draw a polynomial curve (R2≥0.99) to obtain the calculation formula for sample calculation.



3. The sample measurement operation table is as follows:

	Determination wells	**Control wells**
Reagent 1 (μL) pre-warmed at 37 °C	20	20
Sample to be tested (μL)	5	--
37 ℃ for 30 minutes (when taking a sample from each well, insert the nozzle into the matrix solution at the bottom of the well plate, repeatedly pipette and mix, but be careful not to inhale bubbles)		
Reagent 2 (μL)	20	20

Sample to be tested (μL)	--	5
37 ℃ for 20 minutes (when taking a sample from the control well, put the nozzle into the liquid at the bottom of the well plate and mix it repeatedly , but be careful not to suck in bubbles)		
Reagent 3 working solution (μL)	200	200
Gently shake the 96-well plate horizontally to mix, and place it at room temperature for 15 minutes. Use a microplate reader to measure the OD value of each well at a wavelength of 510 nm. Use the absolute OD value (OD value of the measured well minus OD value of the control well) to check the standard curve and obtain the corresponding ALT/GPT activity unit.		

Result calculation

Standard fitting curve: $y = ax^2 + bx + c$

International unit definition: One unit is the amount of enzyme required to catalyze the reduction of 1 μmol NADH per minute at 25°C.

The calculation formula of ALT concentration in serum (plasma) and cell supernatant is:

ALT content (IU/L) = $[a \times (\Delta A510)^2 + b \times \Delta A510 + c] \times 0.482$ IU/L* × f

The calculation formula of ALT concentration in cells and tissues is:

ALT content (IU/gprot) = $[a \times (\Delta A510)^2 + b \times \Delta A510 + c] \times 0.482$ IU/L* × f ÷ C_{pr}

annotation:

y: Karman's unit (0, 28, 57, 97, 150, 200)

x: Standard OD value - Blank OD value (OD value when the Karman unit is 0)

a, b, c: constants corresponding to the fitting curve

ΔA510: Absolute OD value of the sample (sample measurement OD value - sample control OD value)

*: At 25°C, one Karman's unit = 0.482 IU/L

f: dilution factor of the sample before adding it to the detection system

Cpr: tissue sample protein concentration: gprot/L

Notes

1. The commonly used colorimetric methods are the Reitman-Frankel method and the King method. The unit number of the standard curve of the Reitman-Frankel method is obtained by comparing the experimental method with the Karman spectrophotometry (rate method). Reporting the results in Karman units is more accurate.
2. **Definition of Karman's unit:** 1mL liquid, total reaction volume 3mL, wavelength 340nm, 1cm light path, 25℃, pyruvate generated within 1min, oxidizes NADH to NAD+, and causes a

decrease in absorbance of 0.001, which is one unit (1 Karman's unit = 0.482 U/L, 25℃).

3. Generally, serum samples contain very little endogenous ketoacids, and the absorbance value of the serum control well is close to that of the reagent blank well (replace serum with double distilled water, and perform the same operation as the control well). Therefore, when batching samples, it is generally not necessary to make a serum control well for each sample, and the reagent blank well can be used instead. However, for lipemia, icteric or hemolytic serum, each sample should be made into a control well.
4. When the enzyme activity exceeded 150 Karmen's units, the serum was diluted with saline and retested.
5. The absorbance of the control well (or specimen blank well) of general serum should be used as one of the indicators of daily quality control; if there is a large difference, it may be caused by factors such as α-ketoglutaric acid concentration, DNPH concentration and instrumentation.
6. ALT in serum can be stored at room temperature (25°C) for 2 days, at 0-4°C for one week, and at -25°C for 1 month.
7. This product is limited to scientific research by professionals and must not be used for clinical diagnosis or treatment, used as food or medicine, or stored in ordinary residences.