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Aspartate Aminotransferase (AST/GOT) Activity Assay Kit

Catalog No.: BC00058

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

Tel: 0086-27-87002838

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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	Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
Detection Methods	Colorimetric
Sample type	Serum, plasma, tissue, cells
Detection Type	Enzyme activity
Detection instrument and wavelength	Microplate reader (510 nm)
Range	1.10-72.30 IU/L
Sensitivity	1.1 IU/L

Product Introduction

Aspartate aminotransferase (AST), also known as aspartate aminotransferase (GOT), is an enzyme widely found in animals, plants, microorganisms and cultured cells. It catalyzes the transamination reaction between aspartate and α-ketoglutarate, which is crucial in amino acid metabolism. Clinically, AST/GOT is often used as an auxiliary diagnostic indicator for myocardial infarction and myocarditis. At the same time, its concentration in serum will also increase when the liver is damaged.

Detection principle

AST/GOT can transfer the amino and keto groups of ketoglutaric acid and aspartic acid to generate glutamic acid and oxaloacetic acid. Oxaloacetic acid can decarboxylate to pyruvic acid during the reaction. Pyruvic acid reacts with 2,4-dinitrophenylhydrazine to generate 2,4-dinitrophenylhydrazone, which is reddish brown in alkaline solution. After colorimetry, the enzyme activity unit can be obtained by checking the standard curve.

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:

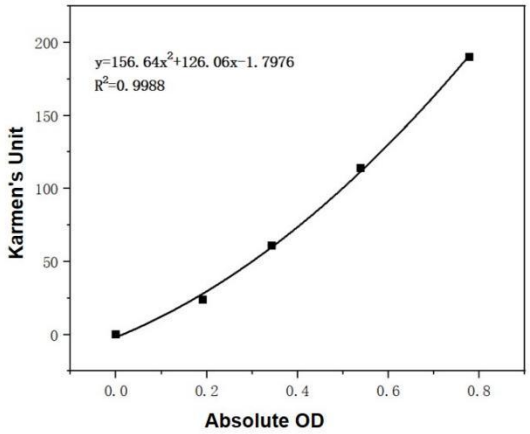
Serial Number	0	1	2	3	4
Reagent 5 (μL)	5	5	5	5	5
Reagent 4 (μL)	0	2	4	6	8
Reagent 1 (μL)	2 0	1 8	1 6	1 4	1 2
Reagent 2 (μL)	2 0	2 0	2 0	2 0	2 0
After mixing, incubate at 37 °C for 20 minutes.					
Reagent 3 working solution (μL)	2 00	2 00	2 00	2 00	2 00

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	0.446	0.638	0.789	0.985	1.224
Absolute OD value	0	0.192	0.343	0.539	0.778
Karman's unit	0	24	61	114	190

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	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 AST/GOT 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 AST concentration in serum (plasma) and cell supernatant is:

A S T content (IU/L) = $[a \times (\Delta A_{510})^2 + b \times \Delta A_{510} + c] \times 0.482$ IU/L* × f

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

A S T content (IU/gprot) = $[a \times (\Delta A_{510})^2 + b \times \Delta A_{510} + c] \times 0.482$ IU/L* × f ÷ C_{pr}

Annotation:

y: Karman's unit (0, 24 , 61 , 114 , 190)

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

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

ΔA_{510} : 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 little endogenous pyruvic acid, 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. AST 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.