Aspartate Aminotransferase (AST/GOT) Activity Assay Kit

Catalogue No.: K098

Size: 96T(43 samples)

Range: 1.10-72.30IU/L

Sensitivity: 1.10IU/L

Kit component:

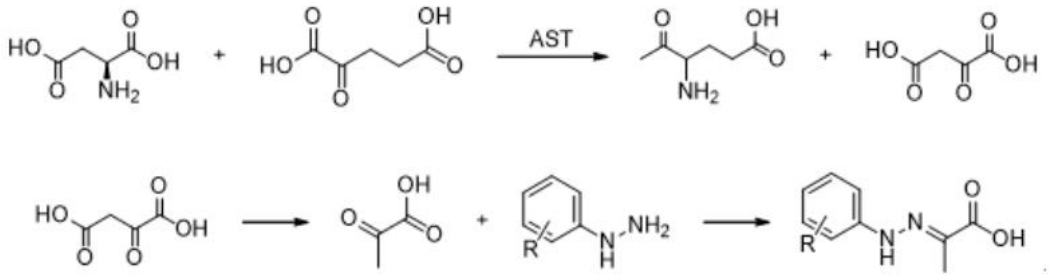
Item	Component	Size（96T）	Storage
Reagent 1	Buffer Solution	0.5 mL	2-8℃
Reagent 2	2 mmol/L Sodium Pyruvate	0.5 mL	2-8℃
Reagent 3	Substrate Solution	5 mL	2-8℃
Reagent 4	Chromogenic Agent	5 mL	2-8℃ shading light
Reagent 5	Alkali Reagent	5 mL	2-8℃
	Product Description	1 copy	

Storage:

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

Principle of the Assay:

AST/GOT enables alpha-ketoglutaric acid and aspartic acid to displace amino and keto groups to form glutamic acid and oxaloacetic acid. Oxaloacetic acid can decarboxylate itself to form Pyroracemic acid during the reaction. Pyroracemic acid reacted with 2,4-dinitro phenyl hydrazine(DNPH) to form 2,4-dinitrophenylhydrazone showing reddish brown in alkaline solution. Measure the OD values and calculate the enzyme activity.



This kit can be used to measure Aspartate Aminotransferase (AST/GOT) 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 1.10-72.30 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
HC-60 cellular supernatant	1
Calu-3 cellular supernatant	1
10% Rat liver tissue homogenization	15-30
10% Rat lung tissue homogenization	2-8

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

③ Add 20 μL of 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.

Calculation:

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard A) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration (0, 24, 61, 114, 190) as x-axis and y-axis respectively.

Create the standard curve (**$y = ax^2 + bx + c$**) with graph software (or EXCEL).

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: 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).

The sample:

1. Serum (plasma) sample:

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

2. Tissue and cells sample:

AST/GOT activity (IU/gprot) = $[a \times (\Delta A_{510})^2 + b \times \Delta A_{510} + c] \times 0.482 \times f \div C_{pr}$

Note:

ΔA_{510}: OD_{sample} - $OD_{control}$

f: Dilution factor of sample before tested.

C_{pr}: 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.