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Glutamate Oxaloacetate Transaminase/Aspartate Aminotransferase (GOT/AST) Activity Assay Kit (Karman method)

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(GOT/AST) Activity Assay Kit (Karman method)

Note: Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer

Cat No: AK1049-50T-48S

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 55 mL×1	2-8℃
Reagent II	Powder×1	-20℃
Reagent III	Liquid 7.5 mL×1	2-8℃

Solution Preparation:

1. Preparation of Reagent I working liquid: Dissolve all the Reagent II in Reagent I, the working liquid of Reagent I is obtained. It can be stored at 2-8℃ for 4 weeks (or stored at -20℃ for 8 weeks after subassembly).

Product Description:

Glutamate Oxaloacetate Transaminase (GOT, EC 2.6.1.1) is also known as Aspartate Aminotransferase (AST). This enzyme is primarily found in tissues such as the heart, liver, and kidneys, where it catalyzes the amino transfer reaction between L-aspartate and α-ketoglutarate, playing a crucial role in amino acid metabolism and energy production. In clinical practice, serum GOT testing serves as a key indicator for evaluating liver function and myocardial injury.

GOT catalyzes the conversion of L-aspartic acid and α-ketoglutarate to oxaloacetate and L-glutamate. Under conditions where malate dehydrogenase and NADH are present, oxaloacetate is reduced to L-malic

acid while NADH is oxidized to NAD+, resulting in a decrease in absorbance at 340nm. This kit calculates GOT activity by measuring the rate of absorbance reduction at 340nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer, water bath/constant temperature incubator/metal bath, mortar/homogenizer/cell ultrasonic crusher, low-temperature centrifuge, adjustable pipette, 1mL quartz cuvette, ice and distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue: Add extract solution according to the ratio of tissue mass (g) : Extract solution volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Extract solution), after ice bath homogenization, centrifuge at 4℃, 8000g for 10min, take supernatant and placed on the ice for test.

2. Bacteria or cell: The ratio of bacteria/cell amount (106): volume of Extract solution (mL) is 5~10:1(it is suggested to take about 5 million bacteria/cells and add 1mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3s, interval 6s, totally 3min). Centrifuge at 8000g for 10min at 4℃, take the supernatant and placed on the ice for test.

3. Serum (plasma) sample: Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure).

II. Determination procedure

1. Preheat ultraviolet spectrophotometer for more than 30min, adjust the wavelength to 340nm, set zero with distilled water.

2. Restore the Reagent I working liquid and Reagent II to normal temperature before use.

3. Operation table (Add the reagents in order in a 1.5mL EP tube or 1mL quartz cuvette)

Reagent Name(μL)

Test Tube(T)

Blank tube(B)

Sample

30

-

Distilled water

-

30

Reagent I working liquid

850

850

Mix thoroughly and incubate at 37℃ for 5min.

Reagent III

120

120

Mix thoroughly and the mixture is placed at 37℃ for 1min. Detect the absorbance value of each tube at

340nm. Record as A1T, A1B. After accurately react at 37℃ for 10min, detect the absorbance value at 340nm. Record as A2T, A2B. ΔAT=A1T-A2T, ΔAB=A1B-

A2B, ΔA=ΔAT-ΔAB. For each batch of samples, the blank tube only need to be tested 1-2 times.

Note: In order to ensure the accuracy and stability of experimental results, please strictly control the operation time and reaction time.

III. Calculation:

1. Calculate by liquid volume

Enzyme activity unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1μmol of NADH in the reaction system per minute every litre volume at

37℃.

GOT activity (U/L)=[ΔA÷(ε×d)×106×VT]÷VS÷T×F=0.5359×ΔA×F

2. Calculate by sample protein concentration:

Enzyme activity unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1μmol of NADH in the reaction system per minute every gram protein at

37℃.

GOT activity (U/g prot)=[ΔA÷(ε×d)×106×VT]÷(Cpr×VS)÷T×F=0.5359×ΔA÷Cpr×F

3. Calculate by sample mass

Enzyme activity unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1μmol of NADH in the reaction system per minute every gram tissue at

37℃.

GOT activity(U/g mass)=[ΔA÷(ε×d)×106×VT]÷(W×VS÷VE)÷T×F=0.5359×ΔA÷W×F

4. Calculate by the number of bacteria or cells

Enzyme activity unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1μmol of NADH in the reaction system per 106 cells or bacteria at 37℃.

GOT activity (U/106 cell)=[ΔA÷(ε×d)×106×VT]÷(N×VS÷VE)÷T×F=0.5359×ΔA÷N×F ε: Molar extinction coefficient of NADH, 6.22×103 L/(mol·cm);

d: 1mL quartz cuvette light path, 1cm;

106: Reduction coefficient, 1mol=106μmol;

VT: Total reaction volume, 1×10-3L;

VS: Sample volume, 3×10-5L;

Cpr: Sample protein concentration, g/L;

W: Sample mass, g;

VE: Extract solution volume, 0.001L;

N: The number of bacteria or cells, count by 106;

T: Reaction time, 10min;

F: Dilution factor.

Note:

1. If (A1B-A1T)>0.1 or ΔAT>0.7, the sample should be diluted with Extract solution before retesting. High-fat or jaundice samples have strong light absorption at 340nm. There may be a situation that the substrate is exhausted but the absorbance value at 340nm remains strong. In such cases, the sample should also be diluted

with the extract solution and retested. If ΔA is too small, the measurement time can be appropriately extended. Pay attention to simultaneously extending the blank tube measurement time and modifying the calculation formula.

2. A dilution ratio of 40-80 times is recommended for liver samples and 20-40 times for kidney samples.

Experimental example:

1. Different samples were extracted according to the extraction method, and 1mL quartz cuvette was used for determination according to the operation table, A1B=1.335, A2B=1.318, ΔAB=0.017. The GOT activity of different samples is shown in the following table:

Sample name
Volume/mass/cell number
F
A1T
A2T
ΔAT
ΔA
GOT activity
Unit
Rat plasma
30μL
1
1.358
1.312
0.046
0.029
15.54
U/L
Rat liver
0.103g
40
1.194
0.514
0.680
0.663
137.98
U/g
RAW264.7 cell
10×106
1
1.261

0.594
0.667
0.650
0.035
U/106cel l
Pothos leaves
0.095g
1
1.451
1.280
0.171
0.154
0.87
U/g