EZMeta™ Aspartate Aminotransferase (AST/GOT) Colorimetric Activity Kit

Cat #: D-AKC1420

Size: 96T

Storage: Stored at -20°C for 6 months, protected from light

Product Information

Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria

Assay Principle

Aspartate aminotransferase (AST/GOT) is widely present in animals, plants, microorganisms and cultured cells. It catalyzes the reversible amino reaction and is an important enzyme for amino acid metabolism. Serum AST activity level is an important biochemical indicator of liver damage, which can reflect the severity of liver damage. In addition, AST has the highest content in cardiomyocytes and is generally used as an auxiliary examination for myocardial infarction and myocarditis in clinical practice. EZMeta™ Aspartate Aminotransferase (AST/GOT) Colorimetric Activity Kit provides a simple method for detecting AST/GOT activity in a variety of biological samples such as Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria. In the assay, Aspartate aminotransferase (AST) catalyzes the transamination reaction of α-ketoglutarate and L-aspartic acid at 37°C and pH7.4 to produce glutamic acid and pyruvate; Pyruvate can interact with 2,4-dinitrophenylhydrazine to produce pyruvate phenylhydrazone, which appears brownish red under alkaline conditions and has a characteristic absorption peak at 505 nm. The rate of pyruvate phenylhydrazone increase at 505 nm can reflect AST/GOT activity.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Extraction Buffer	100 mL	-20°C
Reagent Ⅰ	3 mL	-20°C
Reagent Ⅱ	3 mL	-20°C, protected from light
Reagent Ⅲ	25 mL	-20°C
Standard	1 mL	-20°C

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at OD505 nm

·Incubator, ice maker, refrigerated centrifuge

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·Deionized water

·Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C, protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.

1. Animal Tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Plant Tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

3. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation, add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

4. Plasma and Serum: Tested directly.

5. Standard operation: Mix the standard product and reagent Ⅰ according to the following table.

Standard (μL)	Reagent Ⅰ (μL)	Standard concentration (μmol/mL)
22.5	7.5	1.5
15	15	1
12	18	0.8
6	24	0.4
3	27	0.2
1.5	28.5	0.1
0.75	29.25	0.05
0	30	0

Note: If the protein concentration of the sample is need to determined, it is recommended to use EZMeta™ Aspartate Aminotransferase (AST/GOT) Colorimetric Activity Kit to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement (add the following reagents in sequence into the 96-well plate or microglass cuvette):

Reagent	Test well (μL)	Control well (μL)	Standard Well (μL)
Sample	5	0	0
Reagent I	25	25	0
Different Concentration of Std.	0	0	30
Mix well and heat at 37°C (mammals) or 25°C (other species) for 30 min			
Reagent Ⅱ	25	25	25
Sample	0	5	0
Mix well and heat at 37°C (mammals) or 25°C (other species) for 20 min			
ReagentⅢ	240	240	240

3. Mix well and then let stand for 10 min. Immediately measure at OD505 nm to read as OD_{Test}, $OD_{Control}$, $OD_{Standard}$. Finally, calculate $\Delta OD_{Test} = OD_{Test} - OD_{Control}$, $\Delta OD_{Standard} = OD_{Standard} - OD_{Blank}$.

Note: Every Sample needs to set a Control Tube. The 0 μmol/mL standard well is a Blank well.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

Take the concentration of each standard as the y-axis and $\Delta OD_{Standard}$ as the x-axis, draw a standard curve. Substitute the ΔOD_{Test} into the equation to obtain the y value (μmol/mL).

1. Calculation of GOT activity in Serum (Plasma)

Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 μmol PA per hour per mL of sample.

$GOT(U/mL) = y \times (V_{Sample} + V_{Reagent\ I}) \div V_{Sample} \div T$**$=12y$**

2. Calculation of GOT activity in Tissues, Bacteria or Cells

(1) Calculated by protein concentration

Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 μmol PA per hour per mg of sample.

GOT(U/mg prot)=y×(V_{Sample}+$V_{Reagent\ I}$)÷(Cpr×V_{Sample})÷T=**12y÷Cpr**

Note: The protein concentration of the sample is need to determined.

(2) Calculated by fresh weight of samples

Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 μmol PA per hour per g of sample.

GOT(U/g fresh weight) = y×(V_{Sample}+$V_{Reagent\ I}$)÷(W×V_{Sample}÷$V_{Sample\ Total}$)÷T=**12y÷W**

(3) Calculated by bacteria or cell numbers

Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 μmol PA per hour per 10^4 of sample number.

GOT(U/10^4)=y×(V_{Sample}+$V_{Reagent\ I}$)÷(500÷V_{Sample}÷$V_{Sample\ total}$)÷T=**0.024y**

Where: V_{Sample}: sample volume added, 0.005 mL; $V_{Reagent\ I}$: Reagent Ⅰ volume added, 0.025 mL; $V_{Sample\ Total}$: Extract Buffer added to samples, 1 mL; W: sample weight, g; Cpr: sample protein concentration, mg/mL; T: reaction time, 0.5 h; 500: Total number of bacteria or cells, 5×10^6.

Typical Data

Typical standard curve:

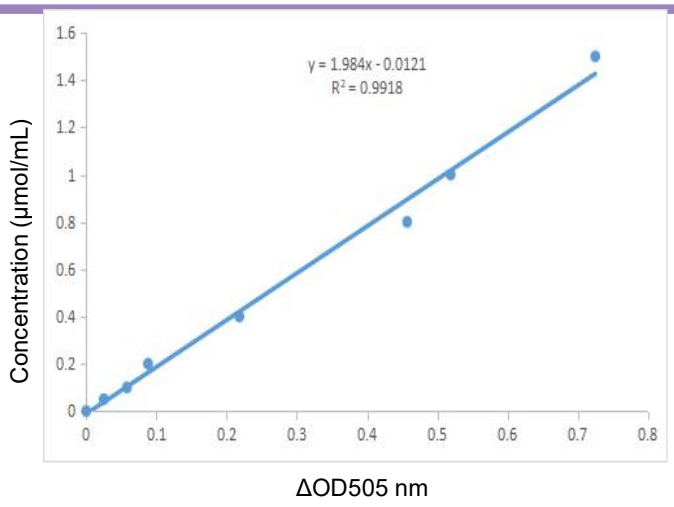

Figure 1. Standard curve for GOT

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.