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GENCHEM® Aspartate Aminotransferase, AST/GOT Activity Assay Kit

REF : KBCA1144

Ver 1.0

RUO

Biochemical Assay for the Quantitative Determination of Aspartate Aminotransferase, AST/GOT Activity in Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria, Urine

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBCA1144 Σ 96 tests

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Introduction:

The GENCHEM® Assay kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma, tissue cells and cell culture supernatant as validated with the kit. The kit employs in vitro quantitative determination of Aspartate Aminotransferase, AST/GOT Activity in the sample.

Intended Use:

The GENCHEM® Aspartate Aminotransferase, AST/GOT Activity Assay kit is used as an analytical tool for quantitative determination of Aspartate Aminotransferase, AST/GOT Activity in Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria, Urine.

Principle:

Glutamic-oxalacetic Transaminase (GOT), also known as Aspartate Aminotransferase (AST), is widely distributed in animals, plants, microorganisms, and cultured cells. It catalyzes reversible transamination reactions and plays an important role in amino acid metabolism. Serum GOT/AST activity is an important biochemical indicator of liver injury and can reflect its severity. Additionally, AST is highly abundant in cardiac muscle cells and is commonly used as an auxiliary marker in the clinical assessment of myocardial infarction and myocarditis. This kit provides a simple method for measuring GOT/AST activity in various biological samples, including animal tissues, plant tissues, adherent and suspension cell cultures, serum, and bacteria. In this assay, GOT/AST catalyzes the transamination reaction between α-ketoglutarate and L-aspartic acid at 37°C and pH 7.4, producing glutamic acid and oxaloacetate. The oxaloacetate is subsequently converted to pyruvate, which reacts with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form pyruvate phenylhydrazone. Under alkaline conditions, this compound develops a brownish-red color and exhibits a characteristic absorption peak at 505 nm. The increase in absorbance at 505 nm reflects the GOT/AST activity in the sample.

Materials Provided:

1. Extraction Buffer – 100 ml
2. Standard (10 umol/ml Pyruvate) – 1 ml
3. Reagent I – 3 ml
4. Reagent II – 3 ml
5. Reagent III – 30 ml

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 505 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Clean tubes and Eppendorf tubes.
6. Precision single and multi-channel pipette and disposable tips.

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. Storage condition for each component has been mentioned on the label, store reagent I at -20°C.
3. All the reagents should be used within 12 months from manufacturing date.
4. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample preparation:

1. Animal and Plant Tissues: Weigh 0.1 g tissue, add 1ml 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. 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.
3. Plasma and Serum: Take 0.1 ml Plasma and Serum, add 1 ml Extraction Buffer and mix well. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Note for sample:

1. If the protein concentration of the sample is need to determined, it is recommended to use BCA Assay.

Reagent preparation:

1. **Extraction Buffer:** Ready to use as supplied. Store at 4°C.
2. **Reagent I:** Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.
3. **Reagent II:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.
4. **Reagent III:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
5. **Standard operation:** Mix the standard and reagent I according to the following table to obtain a standard tube of corresponding concentration.

Setting of Standard Curves: Further dilute the 10 umol/ml standard to 1.5, 1, 0.8, 0.4, 0.2, 0.1, 0.05 umol/ml with Deionized Water, as shown in the following table.

Standard Concentration	Standard Vial	Dilution Particulars
1.5 umol/ml	Standard No.6	60 ul Stock (10 umol/ml) + 340 ul Deionized Water
1 umol/ml	Standard No.5	40 ul Stock (10 umol/ml) + 360 ul Deionized Water
0.8 umol/ml	Standard No.4	32 ul Stock (10 umol/ml) + 368 ul Deionized Water
0.4 umol/ml	Standard No.3	16 ul Stock (10 umol/ml) + 384 ul Deionized Water
0.2 umol/ml	Standard No.2	8 ul Stock (10 umol/ml) + 392 ul Deionized Water
0.1 umol/ml	Standard No.1	4 ul Stock (10 umol/ml) + 396 ul Deionized Water
0.05 umol/ml	Standard No.0	2 ul Stock (10 umol/ml) + 398 ul Deionized Water

Assay Procedure:

1. Preheat the microplate reader or Visible Spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm, use deionized water to set zero Visible Spectrophotometer.
2. Transfer 25 ul of the sample to be tested into an EP tube and boil for 10 min to prepare the control tube.
3. Add the following reagents to the EP tube:

Reagent	Test well (ul)	Control well (ul)	Standard well (ul)	Blank well (ul)
Sample	25	0	0	0
Samples to be tested after boiling for 10 minutes	0	25	0	0
Reagent I	25	25	25	25
Deionized Water	0	0	0	25
Different Concentration of Std.	0	0	25	0

After mixing thoroughly, incubate precisely for 30 min at 37°C for mammalian species or 25°C for other species.				
Reagent II	25	25	25	25
After mixing thoroughly, incubate precisely for 20 min at 37°C for mammalian species or 25°C for other species				
Reagent III	240	240	240	240

4. Pipette up and down repeatedly to mix thoroughly, then incubate at room temperature for 10 min. Centrifuge at 4,000 × g at 25°C for 10 min. Transfer 200 ul of the reaction mixture into a 96-well plate or micro cuvette, and measure the absorbance (A) of each well at 505 nm

5. Calculate the relative absorbance values as follows:

$\Delta A_{(sample)} = A_{(sample)} - A_{(control)}$

$\Delta A_{(standard)} = A_{(standard)} - A_{(blank)}$

(The blank and standard curve only needs to be prepared once.)

Note for Assay Protocol:

1. **The Blank Well only need to measure 1 times. Every Sample needs to set a Control Tube.**
2. **In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.005, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with Deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.**

Data analysis:

1. **Drawing of standard curve:**
Take the concentration of each standard as the y-axis and the $\Delta OD_{Standard}$ as the x-axis, draw a standard curve. Sustitute the ΔOD_{test} into the equation to obtain the y value (umol/ml).

2. **Calculation of GOT/AST activity in sample:**

i. Calculated by fresh weight of samples:
Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 umol PA per hour per g of sample.

$GOT/AST\ (U/g\ fresh\ weight) = y \times V_{Total} \div (W \times V_{Sample} \div V_{Sample\ Total}) \div T = 4y \div W$

ii. Calculated by Bacteria or Cell numbers:
Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 umol PA per hour per 10^4 of sample numbers.

$GOT/AST\ (U/10^4 cells) = y \times V_{Total} \div (500 \times V_{Sample} \div V_{Sample\ total}) \div T = 0.008y$

iii. Calculated by protein concentration
Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 umol PA per hour per milligram of protein.

$GOT/AST\ (U/mg\ prot) = y \times V_{Total} \div (Cpr \times V_{Sample}) \div T = 4y \div Cpr$

iv. Calculated by volume of liquid samples:
Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1 umol PA per hour per ml of liquid sample.

$$GOT/AST(U/ml) = y \times V_{Total} \div (V_{Liquid} \times V_{Sample} \div V_{Sample\ total}) \div T = 4y \div V_{Liquid}$$

Where:
V_{Total}: Total volume of reaction system, 0.05 ml.
V_{sample}: add sample volume, 0.025 ml.
$V_{sample\ Total}$: Add Extraction Buffer volume to sample, 1 ml.
W: sample weight, g.
V_{Total}: add volume of liquid sample, ml.
Cpr: sample protein concentration, mg/ml.
T: reaction time, 0.5 h.
500: Total number of bacteria or cells, $5x10^6$

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Source materials maybe derived from body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

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