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GENCHEM® Alanine Aminotransferase, ALT/GPT Assay Kit

REF : KBCA1051

Ver 1.0

RUO

Biochemical Assay for the Quantitative Determination of Alanine Aminotransferase, ALT/GPT 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 KBCA1051 Σ 96 tests

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Introduction:

Alanine Aminotransferase (ALT), also known as Glutamic-Pyruvic Transaminase (GPT), is an important enzyme involved in amino acid metabolism. It catalyzes the reversible transfer of an amino group from L-alanine to α-ketoglutarate, producing pyruvate and glutamate. ALT is widely distributed in animal and plant tissues, microorganisms, and cultured cells. Serum ALT activity is an important biochemical indicator of hepatocellular injury and is commonly used to assess liver damage. The GPT/ALT Activity Assay Kit provides a convenient method for determining ALT activity in various biological samples, including animal tissues, plant tissues, adherent and suspension cells, serum, and bacteria. The assay is based on the enzymatic conversion of α-ketoglutarate and alanine into glutamate and pyruvate. The pyruvate produced reacts with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form pyruvate phenylhydrazone, which develops a brownish-red colour under alkaline conditions. The colour intensity is measured spectrophotometrically at 505 nm and is proportional to the amount of pyruvate formed, thereby enabling the determination of GPT/ALT activity in biological samples.

Intended Use:

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

Principle:

Glutamic-pyruvic Transaminase (GPT) / Alanine Aminotransferase (ALT) 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 GPT/ALT activity level is an important biochemical indicator of liver damage, which can reflect the severity of liver damage. In addition, GPT/ALT has the highest content in cardiomyocytes, and is generally used as an auxiliary examination for myocardial infarction and myocarditis in clinical practice. This kit provides a simple method for detecting GPT/ALT activity in a variety of biological samples such as Animal Tissues, Plant Tissues, Cell culture (Adherent or Suspension Cells), Serum, Bacteria. In the assay, GPT/ALT catalyzes the transamination reaction of α-ketoglutarate and alanine to produce Glutamic Acid and Pyruvate; Pyruvate can react with 2,4-dinitrophenylhydrazine to form Pyruvate Phenylhydrazone, which appears brownish red under Alkaline conditions and has a characteristic absorption peak at 505nm. The rate of Pyruvate Phenylhydrazone increase at 505 nm can reflect GPT/ALT activity.

Materials Provided:

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

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 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.
3. All the reagents should be used within 12 months from manufacturing date.

4. After receiving the kit, an unopened kit can be stored at -20ºC for 12 months, protected from light. 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 1 ml Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4ºC. Use supernatant for assay, and place it on ice to be tested.

2. **Cells or Bacteria:** Collect $5x10^6$ 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 4ºC. 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 4ºC. 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:

Extraction Buffer: Ready to use as supplied. Store at 4ºC.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at -4ºC.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at -4ºC, protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4ºC.

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.

	Volume of 10 umol/ml Standard (ul)	Volume of Deionized Water (ul)	Standard Concentration (umol/ml)
Std.1	60 ul	340	1.5
Std.2	40 ul	360	1
Std.3	32 ul	368	0.8
Std.4	16 ul	384	0.4
Std.5	8 ul	392	0.2
Std.6	4 ul	396	0.1
Std.7	2 ul	398	0.05

Assay Procedure:

1. Preheat the Microplate Reader or Visible Spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm. Visible Spectrophotometer deionized water zero.

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

Reagent	Test well (ul)	Control well (ul)	Standard well (ul)	Blank well (ul)
Reagent I	25	25	25	25
Sample	25	0	0	0
Deionized water	0	0	0	25
Different concentration of standard	0	0	25	0
Mix well, incubate for 30 min at 37ºC (mammal) or 25ºC (Other species)				
Reagent II	25	25	25	25
Sample	0	25	0	0
Mix well, let stand at room temperature for 2 min				
Reagent III	125	125	125	125

Mix well with precision Pipette, measure at 505 nm to read as OD_{Test}, $OD_{Control}$, $OD_{Standard}$, OD_{Blank}. Finally, calculate $\Delta OD_{Test} = OD_{Test} - OD_{Control}$, $\Delta OD_{Standard} = OD_{Standard} - OD_{Blank}$.

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 1.2 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 $\Delta OD_{Standard}$ as the x-axis, draw a standard curve. Substitute the ΔOD_{Test} into the equation to obtain the y value (umol/ml).

2. Calculation of GPT/ALT activity in Sample:

(1) Calculated by fresh weight of samples
Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1umol PA per hour per g of sample.
GPT/ALT (U/g fresh weight) = $y \times V_{Total} \div (W \times V_{Sample} \div V_{Sample\ Total}) \div T = 4y \div W$

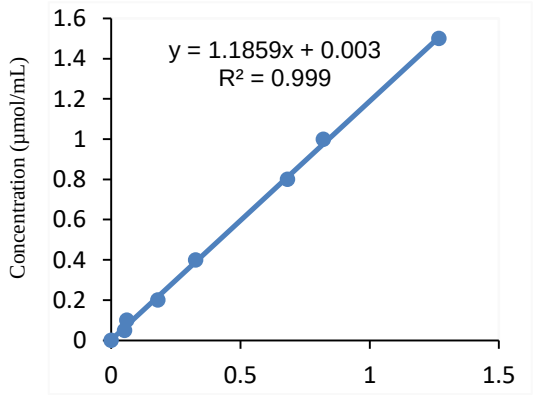
(2) Calculated by Bacteria or Cell numbers
Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1umol PA per hour per 10^4 of sample numbers.
GPT/ALT ($U/10^4$cells)$= y \times V_{Total} \div (500 \times V_{Sample} \div V_{Sample\ total}) \div T = 0.008y$

(3) Calculated by protein concentration
Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1umol PA per hour per milligram of protein.
GPT/ALT (U/mg prot)$= y \times V_{Total} \div (Cpr \times V_{Sample}) \div T = 4y \div Cpr$

(4) Calculated by volume of liquid samples
Active unit definition: One unit defines as the amount of enzyme that catalyzes and generates 1umol PA per hour per ml of liquid sample.
GPT/ALT(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, 0.025ml; $V_{Sample\ total}$: add Extraction Buffer volume to sample, 1 ml; W: weight of sample, 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, 5×10^6.

Typical standard curve:



ΔOD505 nm

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.

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