Alanine Aminotransferase (ALT/GPT) Assay Kit

Catalog No.: abx092105

Size: 100 Assays

Detection Range: 1.26 IU/L – 72.3 IU/L

Sensitivity: 1.26 IU/L

Storage: Store the Detection Reagent in the dark at 2-8°C.Store the rest of the kit components at 2-8°C.

Application: For measurement of Alanine Aminotransferase activity in serum, plasma, tissue, cell culture supernatant, urine and other biological samples.

Introduction

Alanine Aminotransferase (ALT) is a transaminase enzyme and is also known as Alanine Transaminase (ALAT) or Serum Glutamate-Pyruvate Transaminase/Serum Glutamic-Pyruvic Transaminase (SGPT). Alanine Aminotransferase is found most commonly in the liver where it catalyzes two parts of the Alanine cycle. Common biomarkers for liver health include Serum Alanine Aminotransferase, Serum Aspartate Transaminase and their ratio (AST:ALT). At pH 7.4 and 37°C, Alanine Aminotransferase will catalyze the reaction between α-ketoglutaric acid and alanine to produce glutamic acid and pyruvic acid. Phenylhydrazine will be added to form pyruvic acid and phenylhydrazone which is a reddish-brown under alkaline conditions.

Abbexa's Alanine Aminotransferase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Alanine Aminotransferase activity. The product has an absorbance maxima at 505 nm. The intensity of the color is proportional to Alanine Aminotransferase activity, which can be calculated.

Kit components

1. 96-tube microplate
2. Buffer Solution: 1.8 ml
3. 2 mmol/L Sodium Pyruvate: 1.8 ml
4. Substrate Solution: 2 × 30 ml
5. Detection Reagent: 2 × 30 ml
6. Alkaline Reagent: 2 × 30 ml

Materials Required But Not Provided

1. Spectrophotometer (505 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Alkaline Reagent working solution**: Dilute the Alkaline Reagent with double distilled water at a 1:9 ratio and mix fully. Prepare the solution before use.
- **Substrate Solution**: Incubate at 37°C for 10 minutes before use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles. The homogenized medium is PBS (0.01 M, pH 7.4) including 0.1 mM EDTA.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position for 30 minutes at 25°C. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month. The serum sample can be stored at 2-8°C for up to 7 days and at -20°C for up to 20 days.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue and wash with homogenization medium at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at a 9:1 ratio of the volume of homogenized medium with the weight of the tissue. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 1500 × g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with the homogenization medium 1-2 times. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl of homogenization medium per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 10000 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.
- **Urine:** Collect fresh urine into a sterile container, then centrifuge at 10000 × g at 4°C for 15 min. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat Liver Tissue Homogenization	30-60
10% Rat Kidney Tissue Homogenization	1
Human Serum	1
Human Plasma	1
HepG2 Cell Lysates	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- The sample diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

B. Assay Procedure

1. Set standard, sample and control tubes, and label accordingly. *It is recommended to measure in duplicate.*
2. Add 0.5 ml of pre-heated Substrate Solution to the sample tubes and control tubes.
3. Add 0.1 ml of sample to the sample tubes.
4. Mix fully and incubate the sample and control tubes at 37°C for 30 minutes.
5. Add 0.1 ml of Buffer Solution to the standard tubes.
6. Add 0, 0.05, 0.10, 0.15, 0.20, and 0.25 ml of 2 mmol/L Sodium Pyruvate to the corresponding standard tubes.
7. Add 0.50, 0.45, 0.40, 0.35, 0.30, and 0.25 ml of Substrate Solution to the corresponding standard tubes.
8. Add 0.5 ml of Detection Reagent to all tubes.
9. Add 0.1 ml of sample to the control tubes.
10. Mix fully and incubate at 37°C for 20 minutes.
11. Add 5 ml of Alkaline Reagent working solution to all tubes.
12. Leave for 10 minutes at room temperature. Meanwhile, set the Spectrophotometer to zero with double distilled water at 505 nm.
13. Measure the OD of each tube at 505 nm.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{505} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax^2 + bx + c$). Create the standard curve with graph software. The carmen unit of the sample can be calculated according to the formula based on the OD value.

1) **International unit**: One unit is defined as the amount of enzyme 1 µmol of NADH is consumed in a reaction system per minute, when there is 1 ml sample or 1 g of tissue, temperature at 25 °C, the wavelength is 340 nm and the optical path is 1 cm.
2) **Carmen unit**: One unit is defined as the amount of produced pyruvic acid which oxidizes NADH to NAD+ and decreases absorbance by 0.001 when there is 1 ml of sample, 3 ml of total sample, wavelength is 340 nm, the optical path is 1 cm, temperature at 25°C for 1 minute. (1 Karmen unit = 0.482 IU/L, at 25°C)

1. Serum and plasma samples:

$$\text{ALT/GPT activity (IU/L)} = [\text{a} \times (\Delta A_{505})^2 + c] \times 0.482 \times f$$

2. Tissues and cell lysate samples:

$$\text{ALT/GPT activitiy (IU/gprot)} = [\text{a} \times (\Delta A_{505})^2 + c] \times 0.482 \times \frac{f}{\text{C}_{\text{pr}}}$$

where:

y	Carmen unit of sample
x	$OD_{Standard} - OD_{Blank}$
$a.b,c$	The constants of the standard curve $y = ax^2 + bx + c$
ΔA_{505}	$OD_{Sample} - OD_{Control}$
OD_{Blank}	OD value of where the Carmen unit (y) = 0
f	The dilution factor of the sample before testing
C_{pr}	Concentration of protein in the sample (gprot/L)
0.482	1 Carmen unit = 0.482 IU/L at 25°C