Alanine Transaminase (ALT) Assay Kit

Catalog No.: abx096001

Size: 96 tests

Detection Range: 0.75 IU/L – 72.3 IU/L

Sensitivity: 0.75 IU/L

Storage: Store all components in the dark at 4°C for up to 12 months.

Application: For detection and quantification of ALT activity in serum, plasma, tissue, cell lysates, cell culture supernatants, and other biological fluids.

Introduction

Alanine transaminase (ALT), also known as alanine aminotransferase, is widely found in plasma and various tissues of the body, including liver, kidney, heart, and skeletal muscle. ALT is an important pyridoxal phosphate dependent enzyme in the intermediate metabolism of glucose and protein. Clinically, the activity of serum alanine aminotransferase is often used as a marker for alcoholic liver disease, liver cirrhosis and acute viral hepatitis. This Assay kit uses the Reitman-Frankel Method to quantify the activity of ALT in samples. ALT catalyzes the amino conversion reaction between alanine and α -ketoglutaric acid to produce pyruvic acid and glutamic acid at pH 7.4 and 37°C. Adding phenylhydrazine forms phenylhydrazone with pyruvic acid. By colorimetric analysis of the catalysis products, units of ALT activity can be calculated.

Abbexa's Alanine Transaminase (ALT) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating ALT activity. The phenylhydrazone product has an absorbance maximum at 510 nm, and the intensity of the color is proportional to the activity of ALT.

Kit components

1. 96-well microplate
2. Assay Buffer: 0.5 ml
3. Standard Solution (2 mmol/L): 0.5 ml
4. Substrate Solution: 5 ml
5. Detection Reagent: 5 ml
6. Alkali Reagent: 5 ml
7. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (500-520 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Homogenization medium: PBS (0.01 M, pH 7.4) containing 0.1 mM EDTA
5. Pipette and pipette tips
6. Vials/tubes
7. 37°C Water bath/Incubator
8. Centrifuge
9. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. 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 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 overnight at 4°C or at room temperature for up to 1 hr. 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.
- **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 the tissue homogenate. For each 1 g of homogenate, add 9 ml homogenization medium. 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 PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl homogenization medium per 1 × 10^6 cells, then sonicate in an ice water bath at 200 W in 2 second bursts with a 3 second rest interval, for 5 minutes in total. Centrifuge at 1500 × 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 and centrifuge at 10,000 × g for 15 min at 4°C. Take the supernatant, place on ice and assay immediately.

Sample dilution ratios

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Samples should be diluted using Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). The reference dilution factors for different sample types follows (for reference only):

Sample type	Dilution factor
Human Serum	1
Human Plasma	1
Pig Serum	1
Rat Serum	1
10% Rat Brain Tissue Homogenate	1
10% Rat Heart Tissue Homogenate	1
10% Rat Liver Tissue Homogenate	40-60
10% Rat Kidney Tissue Homogenate	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.

2. Reagents

- **Alkali Reagent Working Solution:** Dilute the Alkali Reagent with double-distilled water to a ratio of 1:9. Prepare immediately before carrying out the assay.
- **Substrate Solution Working Solution:** Incubate the Substrate Solution at 37°C for 10 minutes prior to use.

B. Assay Procedure

1. Set the Standard, Sample and Sample Control wells, and record their positions. Label the standard wells 0, 28, 57, 97, 150, 200 U respectively. *Each sample well requires a sample control well.*
2. Add 5 µl of Assay Buffer to each standard well.
3. Add the following volumes of Substrate Working Solution to each Standard well: 20, 18, 16, 14, 12, 10 µl.
4. Add the following volumes of Standard Solution to each Standard well: 0, 2, 4, 6, 8, 10 µl.
5. Heat the Substrate Solution at 37°C for 10 minutes, then add 20 µl of preheated Substrate Solution to each Sample well and each Sample Control well.
6. Add 5 µl of sample to each Sample well.
7. Mix fully using an orbital shaker. Incubate 37°C for 30 minutes.
8. Add 20 µl of Detection Reagent to all wells.
9. Add 5 µl of sample to each Sample Control well. Mix fully with an orbital shaker.
10. Mix fully with an orbital shaker for 10 seconds. Incubate 37°C for 20 minutes.
11. Add 200 µl of Alkali Reagent working solution to all wells.
12. Mix full with an orbital shaker for 10 seconds. Allow to stand at room temperature for 15 minutes.
13. Measure the OD values at 510 nm with a microplate reader.

C. Calculation of Results

One International Unit (IU) of activity is defined as the quantity of ALT in 1 ml or 1 g of sample that catalyzes the consumption of 1 µmol/L NADH at 25°C per minute.

One Carmen Unit of activity is defined as the quantity of ALT in 1 ml or 1 g of sample that causes a decrease in OD of 0.001, catalyzing the consumption of NADH at 25°C per minute (1 Carmen unit = 0.482 IU/L).

The standard curve can be plotted as the **absolute OD_{520} of each standard solution** (*x*) vs. the respective **Carmen unit** (y). A quadratic fit is recommended for the standard curve ($y = ax^2 + bx + c$). The Carmen unit and International Unit activity of the samples can be interpolated from the standard curve.

1. Serum, plasma, and cell culture supernatant samples:

$$\text{ALT (IU/L)} = [a(A_{510})^2 + b(A_{510}) + c(A_{510})] \times f \times 0.482$$

2. Tissue and cell lysates:

$$\text{ALT (IU/g prot)} = [a(A_{510})^2 + b(A_{510}) + c(A_{510})] \times \frac{f \times 0.482}{C_P}$$

where:

y	Carmen unit
x	$OD_{Standard} - OD_{Blank}$
A_{510}	OD absorbance value of the sample measured at 510 nm
a	the quadratic coefficient (quadratic fit: $y = \underline{\mathbf{a}}x^2 + bx + c$)
b	the linear coefficient (quadratic fit: $y = ax^2 + \underline{\mathbf{b}}x + c$)
c	the constant coefficient (quadratic fit : $y = ax^2 + bx + \underline{\mathbf{c}}$)
f	dilution factor of the sample before carrying out the assay
0.482	coefficient of conversion between Carmen Units and International Units
C_P	concentration of protein in sample (mg protein/ml)