Alanine Aminotransferase (ALT/GPT) Activity Assay Kit

Catalog Number EEA001 (96 tests)

Rev 1.0

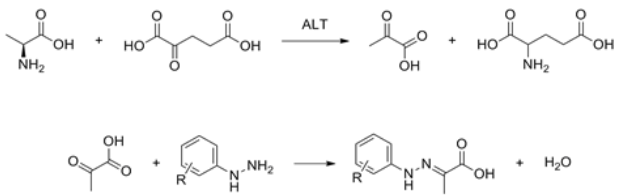
For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can be used to measure ALT/GPT activity in animal serum, plasma, tissue, culture cells and cell culture supernatant.

Alanine aminotransferase (ALT) 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 proteins. Clinically, the activity of serum alanine aminotransferase is often used as a marker for alcoholic liver disease, liver cirrhosis, and acute viral hepatitis.

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 ℃. Then phenylhydrazine is added to form phenylhydrazone with pyruvic acid. Phenylhydrazone is a reddish brown solution under alkaline conditions. ALT activity can be calculated by measuring the OD values at 510 nm.



Contents and storage

Kit and components are shipped at 2-8 ℃. An unopened kit can be stored at 2-8 ℃ for 12 months.

Components	Quantity (96 tests)
Buffer Solution	0.5 mL
2 mmol/L Sodium Pyruvate	0.5 mL
Substrate Solution	5 mL
Chromogenic Agent	5 mL
Alkali Reagent	5 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 510 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37 ℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant for detection. **Urine:** Collect fresh urine and centrifuge at 10000 g for 15 min at 4 ℃. Take the supernatant to preserve it on ice for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with homogenization medium at 2-8 °C to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8 °C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4 ℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80 ℃ for 1 month

Cells:

- Collect the cells and wash the cells with homogenization medium for 1 times.
- Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment.
- Add homogenization medium at a ratio of cell number (10^6): PBS (0.01 M, pH 7.4) =1: 300-500.
- Sonicate or grind with hand-operated in ice water bath.
- Centrifuge at 10000 g for 10 min at 4 °C, then take the supernatant and preserve it on ice for detection

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227). If not detected on the same day, the cells sample (without homogenization) can be stored at -80 ℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before the formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.75-72.3 IU/L).

Note: Use all samples within 2 hours of dilution

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

Sample type	Dilution factor
Human serum	1
Human plasma	1
Porcine serum	1
Rat serum	1
10% Rat brain tissue homogenization	1
10% Rat heart tissue homogenization	1
10% Rat liver tissue homogenization	40-60
10% Rat kidney tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

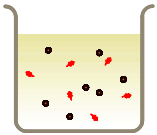
Preparation of alkali reagent working solution

Dilute alkali reagent 1:10 with deionized or distilled water and mix fully. Prepare the fresh solution before use.

Preparation of substrate solution

Incubate at 37 ℃ for 10 min before use.

Assay procedure



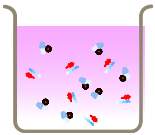
1. Add sample, standard and control

a. **Standard wells**: Add 5 μL of buffer solution to the standard wells (multi-channel pipette is recommended to be used). Add 20, 18, 16, 14, 12, 10 μL of substrate solution to the standard wells from A to F, respectively. Add 0, 2, 4, 6, 8, 10 μL of 2 mmol/L sodium pyruvate to the standard wells from A to F, respectively.

b. **Sample wells**: Add 20 μL of substrate solution (pre-heated at 37 ℃ for 10 min) and 5 μL of sample.

Control wells: Add 20 μL of substrate solution (pre-heated at 37 ℃ for 10 min).

c. Mix fully, then incubate at 37 ℃ for 30 min



2. **Add substrate**

a. Add 20 μL of chromogenic agent to each well.

b. Add 5 μL of sample to the control wells.

c. Mix fully with microplate reader for 10 s, then incubate at 37 ℃ for 20 min.

d. Add 200 μL of alkali reagent working solution to each well (the multi-channel pipette is recommended).

e. Mix fully with microplate reader for 10 s, then let stand for 15 min at room temperature and measure the OD value of each well with the microplate reader at 510 nm.

Calculation

1. Definition of international unit: The enzyme amount of 1 μmol of NADH consumed in reaction system (1 mL sample or 1 g tissue protein, 25 ℃) per minute is defined as 1 unit (wavelength is 340 nm, optical path is 1 cm).

2. Definition of Carmen unit: 1 mL of sample, the total volume of reaction is 3 mL, wavelength is 340 nm, optical path is 1 cm, react at 25 ℃ for 1 min, the amount of generated pyruvic acid which oxidize NADH to NAD^+ and causes absorbance decreasing 0.001 is as 1 unit. (1 Carmen unit = 0.482 IU/L, 25℃)

3. Plot the standard curve by using OD value of standard and correspondent Carmen unit (0, 28, 57, 97, 150, 200 Carmen unit) as x-axis and y-axis respectively. Create the standard curve with graph software (or EXCEL). The Carmen unit of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is $y=ax^2+bx+c$.

Serum (plasma) and other liquid sample:

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

Tissue and cells sample:

$$\text{ALT/GPT activity (IU/gprot)} = [a \times (\Delta A_{510})^2 + b \times (\Delta A_{510}) + c] \times 0.482 \text{ IU/L} \times f \div C_{pr}$$

[Note]

y: Carmen unit.

x: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the carmen unit is 0)

a, b, c: the constant of standard curve.

ΔA_{510}: $OD_{sample} - OD_{control}$

f: dilution factor of sample before tested.

C_{pr}: concentration of protein in sample (gprot/L)

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

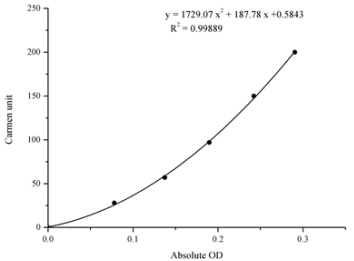
Take 5 μL of rabbit serum, carry the assay according to the operation table. The results are as follows: standard curve: $y = 1729.07 x^2 + 187.78 x + 0.5843$, the average OD value of the sample well is 0.303, the average OD value of the control well is 0.254, the calculation result is:

$$\text{ALT activity(IU/L)} = [1729.07 \times (0.303 - 0.254)^2 + 187.78 \times (0.303 - 0.254) + 0.5843] \times 0.482 = 6.72 \text{ IU/L}$$

Performance characteristics

▪Standard curve (example)

The following data were obtained for the various standards over the range of 0.75-72.3 IU/L standard.

Concentration (Carmen unit)	OD at 510 nm	Standard Curve
200	0.537	
250	0.489	
97	0.436	
57	0.384	
28	0.324	
0	0.247	

Inter-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (Carmen unit)	28.00	57.00	150.00
%CV	9.3	9.2	8.5

CV = Coefficient of Variation

▪Intra-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (Carmen unit)	28.00	57.00	150.00
%CV	5.0	5.6	5.3

CV = Coefficient of Variation

▪Expected values

This assay was tested with serum without dilutions.

Sample Type	Range (IU/L)	Average (IU/L)
Human Serum	9-50	29.5

▪Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (Carmen unit)	28	57	150
Observed Conc. (Carmen unit)	26.04	54.15	147
Recovery rate (%)	93	95	98

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S3'	S3	S11'	S11	S19'	S19	S27'	S27	S35'	S35
B	B	B	S4'	S4	S12'	S12	S20'	S20	S28'	S28	S36'	S36
C	C	C	S5'	S5	S13'	S13	S21'	S21	S29'	S29	S37'	S37
D	D	D	S6'	S6	S14'	S14	S22'	S22	S30'	S30	S38'	S38
E	E	E	S7'	S7	S15'	S15	S23'	S23	S31'	S31	S39'	S39
F	F	F	S8'	S8	S16'	S16	S24'	S24	S32'	S32	S40'	S40
G	S1'	S1	S9'	S9	S17'	S17	S25'	S25	S33'	S33	S41'	S41
H	S2'	S2	S10'	S10	S18'	S18	S26'	S26	S34'	S34	S42'	S42

[Note]: A-F, standard wells; S1'-S42', control wells; S1-S42, sample wells.

▪Sensitivity

The analytical sensitivity of the assay is 0.75 IU/L. This was determined by adding two standard deviations to the mean OD obtained when the zero standard was assayed 20 times and calculating the corresponding concentration.

Limited product warranty

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