

CheKine™ Alanine Aminotransferase (ALT/GPT) Activity Assay Kit (Reitman-Frankel Method)

Cat #: KTB1410

Size: 96 T/48 S

192 T/96 S

	Alanine Aminotransferase (ALT/GPT) Activity Assay Kit (Reitman-Frankel Method)		
REF	Cat #: KTB1410	LOT	Lot #: Refer to product label
	Applicable samples: Serum, Plasma, Animal Tissues, Cells		
	Storage: Stored at 4°C for 24 months, protected from light		

Assay Principle

Alanine Aminotransferase (ALT/GPT) 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. Mammalian hepatocytes exhibit high glutamic-pyruvic transaminase (ALT) activity. When hepatocytes undergo necrosis, ALT is released into the bloodstream, resulting in a marked increase in serum ALT activity. Serum ALT activity level is an important biochemical indicator of liver damage, which can reflect the severity of liver damage. CheKine™ Alanine Aminotransferase (ALT/GPT) Activity Assay Kit (Reitman-Frankel Method) provides a simple method for detecting ALT/GPT activity in a variety of biological samples such as Serum, Plasma, Animal Tissues, Cells. In the assay, ALT catalyzes the transamination reaction of α -ketoglutarate and alanine at 37°C and pH7.4 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 505 nm. The rate of pyruvate phenylhydrazone increase at 505 nm can reflect ALT/GPT activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	192 T	
Reagent I	3.2 mL	6.4 mL	4°C
Reagent II	3.2 mL	6.4 mL	4°C, protected from light
Reagent III	32 mL	64 mL	4°C
Reagent IV	1 mL	1 mL	4°C
Standard	1 mL	1 mL	4°C

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 505 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

- 1×PBS (pH 7.4)
- Homogenizer (for tissue samples), Ultrasonic disruptor (for cells)

Reagent Preparation

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

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

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

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

2 µmol/mL Standard: Ready to use as supplied. Equilibrate to room temperature before use. 2 µmol/mL sodium pyruvate standard solution; Store at 4°C.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C for one month.

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL 1×PBS (pH 7.4) and homogenize or mortar on ice (Recommended parameter: add three 3 mm steel beads, at 55 HZ, homogenize for 30s, with 10-second intervals, and repeat 4 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Cells: Collect 5×10⁶ cells or into the centrifuge tube, wash cells with cold PBS, centrifuge at 800 g for 2 min, and discard the supernatant, add 1 mL 1×PBS (pH 7.4) to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s repeat 30 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

3. Plasma and Serum: Tested directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample. The samples extracted by this kit can also be used for the determination of KTB1420, KTB2302, KTB2303.

Assay Procedure

Note: When preparing the detection system, except for Reagent III, all other reagents should be added to the bottom of the microplate, and all added reagents should be concentrated in a single area; to prevent contamination, it is essential to change the pipette tip. Thoroughly mix the reagents before incubation; failure to mix adequately or accidental contact with the wellside may compromise the detection results. For mouse liver samples, a 100-300× dilution is recommended; for rabbit liver samples, a 20-60× dilution is recommended.

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Preparation of the standard curve detection system: Using a 2 µmol/mL sodium pyruvate standard solution, add the standards sequentially into a 96-well plate according to the table below. For each experiment, perform one standard curve determination to generate the standard curve. The Karmen units corresponding to the blank well, Standard Well 1, Standard Well 2, Standard Well 3, Standard Well 4, and Standard Well 5 are 0, 28, 57, 97, 150, and 200, respectively.

Reagent (µL)	Blank Well	Standard Well 1	Standard Well 2	Standard Well 3	Standard Well 4	Standard Well 5
Reagent IV	5	5	5	5	5	5
Standard	0	2	4	6	8	10
Reagent I	20	18	16	14	12	10
Reagent II	20	20	20	20	20	20

Mix well and heat at 37°C for 20 min.

Reagent III	200	200	200	200	200	200
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3.Preparation of the sample testing system: A control well must be included in each assay well; for 96-well plates, follow the procedure below.

Reagent	Test Well (μL)	Control Well (μL)
Sample	5	0
Reagent I	20	20

Mix well and heat at 37°C for 30 min.

Reagent II	20	20
Sample	0	5

Mix well and heat at 37°C for 20 min.

Reagent III	200	200
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4.Mix well and then let stand for 10 min. The absorbance value is measured at 505 nm. The Blank Well is recorded as A_{Blank} , the standard Well is marked as A_{Standard} , the test Well is marked as A_{Test} , and the control Well is marked as A_{control} . Finally calculate $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{control}}$, $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the y-value obtained from sample testing exceeds 150, the sample may be diluted with 1×PBS (pH 7.4) and retested. If the y-value obtained from sample testing is less than 5, the sample volume may be appropriately increased, and the test may be repeated.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Drawing of standard curve

Using the Karmen units of the blank well and the standard wells as the y-axis, and $\Delta A_{\text{Standard}}$ as the x-axis, a standard curve is plotted to derive the polynomial equation $y = ax^2 + bx + c$. The ΔA_{Test} is then substituted into this equation to obtain the corresponding Karmen unit value y.

Calculation of sample ALT activity

Definition of the Karmen units: At 25°C, for a 1 mL sample, a total reaction mixture volume of 3 mL, a wavelength of 340 nm, a path length of 1 cm, and a reaction duration of 1 min, one Karmen units is defined as the amount of pyruvate generated that causes a decrease in absorbance of 0.001 due to the oxidation of NADH to NAD^+ . (1 Karmen units = 0.482 U/L at 25°C)

Definition of the international unit: One international unit is defined as the amount of enzyme activity in 1 L of sample that catalyzes the consumption of 1 μmol of NADH during a 1 min reaction at 25°C.

1. Calculated by volume of serum (plasma)

$$\text{ALT (U/L)} = \mathbf{0.482y}$$

2. Calculated by protein concentration

$$\text{ALT (U/g prot)} = \mathbf{0.482y \div Cpr}$$

3. Calculated by sample fresh weight

$$\text{ALT (U/g)} = 0.482y \times V_{\text{Total Sample}} \div W = \mathbf{4.82 \times 10^{-4}y \div W}$$

4. Calculated by cell number

$$\text{ALT (U/10}^6) = 0.482y \times V_{\text{Total Sample}} \div N = \mathbf{4.82 \times 10^{-4}y \div N}$$

0.482: 25 °C; 1 Karmen unit = 0.482 U/L (international units); $V_{\text{Total Sample}}$: add 1×PBS (pH 7.4) volume, 0.001 L; Cpr: sample protein concentration, g/L; W: sample weight, g; N: Number of cells, expressed in units of 10^6 (e.g., if the number of cells is 5×10^6 , then $N=5$).

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

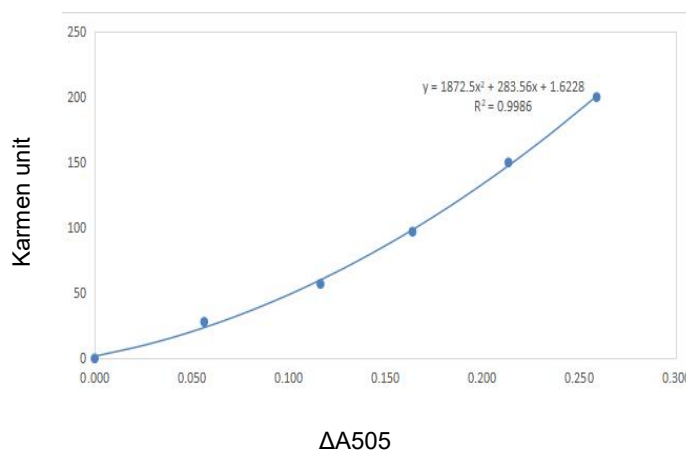


Figure 1. Standard Curve of ALT assay.

Recommended Products

Catalog No.	Product Name
KTB1420	CheKine™ Aspartate Aminotransferase (AST/GOT) Activity Assay Kit (Reitman-Frankel Method)
KTB2302	CheKine™ Alanine Aminotransferase (ALT/GPT) Activity Assay Kit (IFCC Rate Method)
KTB2303	CheKine™ Aspartate Aminotransferase (AST/GOT) Activity Assay Kit (IFCC Rate Method)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.