

CheKine™ Aspartate Aminotransferase (AST/GOT) Activity Assay Kit (Reitman-Frankel Method)

Cat #: KTB1420

Size: 96 T/48 S

192 T/96 S

	Aspartate Aminotransferase (AST/GOT) Activity Assay Kit (Reitman-Frankel Method)		
REF	Cat #: KTB1420	LOT	Lot #: Refer to product label
	Applicable samples: Animal Tissues, Cells, Plasma, Serum		
	Storage: Stored at 4°C for 12 months, protected from light		

Assay Principle

Aspartate Aminotransferase (AST, formerly known as GOT) is an intracellular aminotransferase, widely distributed in the mitochondria and cytoplasm of animal/human tissues including myocardium, liver, skeletal muscle, kidney and others. Its concentration is extremely low in body fluids under physiological conditions; massive enzyme release into the blood occurs upon cellular injury and rupture. AST catalyzes the reversible transamination between L-aspartate and α -ketoglutarate, participating in in vivo amino acid metabolism and gluconeogenesis pathways. In clinical assays, AST activity serves as a critical biomarker for assessing the extent of hepatocyte and cardiomyocyte injury. CheKine™ Aspartate Aminotransferase (AST/GOT) Activity Assay Kit (Reitman-Frankel Method) can be used to detect biological samples such as animal tissues, cells, plasma and serum. Under the conditions of 37 °C and pH 7.4, aspartate aminotransferase (AST/GOT) catalyzes the transamination reaction between α -ketoglutarate and L-aspartate to produce glutamate and pyruvate. Pyruvate reacts with 2,4-dinitrophenylhydrazine to form pyruvate phenylhydrazone, which exhibits a brownish-red color under alkaline conditions. The absorbance is measured at 505 nm, and the AST/GOT enzyme activity can be calculated accordingly.

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

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 505 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, ice maker, freezing centrifuge
- 1×PBS (pH 7.4)
- Homogenizer or mortar (for tissue samples), ultrasonic cell 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.

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 using fresh samples. Samples should be collected as fresh as possible. If collection cannot be performed on the same day, store whole cells or aliquots of tissue at -80°C and use them within one month.

1. Animal tissues: Weigh approximately 0.1 g of tissue, add 1 mL of 1×PBS (pH 7.4). Perform ice-bath homogenization with a pre-cooled tissue grinder (recommended parameters: add 3 steel beads of 3 mm, 55 Hz, homogenize for 30 s, with a 10 s interval, repeat 4 times). Centrifuge at 10,000 g, 4°C for 10 min, transfer the supernatant to an ice bath for subsequent assay.
2. Cells: Collect 5×10^6 cells, wash the cells with cold PBS, centrifuge at 800 g for 2 min, and discard the supernatant.; add 1 mL of 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, 4°C for 10 min, transfer the supernatant to an ice bath for subsequent assay.
3. Plasma, Serum: Test directly.

Note: (1) If the protein concentration of the sample needs to be determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

(2) The samples extracted by this kit can also be applied to the determination of KTB1410, KTB2302 and KTB2303.

Assay Procedure

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 standard curve assay system: Use 2 µmol/mL sodium pyruvate standard solution, and add reagents sequentially into a 96-well plate as shown in the table below. A standard curve shall be generated by running standard samples in each assay. The Karmen units corresponding to blank well, standard well 1, standard well 2, standard well 3 and standard well 4 are 0, 24, 61, 114 and 190, respectively.

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

Mix well, Incubate for 20 min at 37°C

Reagent III	200	200	200	200	200
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3. Sample detection system preparation: A control well shall be set for each test well. Perform the operations in the 96-well plate as described below.

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

Mix well, Incubate for 30 min at 37°C

Reagent II	20	20
Sample	0	5

Mix well, Incubate for 20 min at 37°C

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 marked as A_{Blank} , the standard Well is marked as A_{Standard} , and 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 detection is greater than 150, dilute the sample with 1×PBS (pH 7.4) and re-test. If the y value is less than 3, appropriately increase the sample volume and perform the measurement again. For liver samples, the recommended dilution factor is 20-60-fold.

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.

1. Drawing of standard curve

Plot the standard curve with the Karmen units of blank well and each standard well on the y-axis and $\Delta A_{\text{Standard}}$ on the x-axis, to obtain the polynomial equation $y = ax^2 + bx + c$. Substitute the measured ΔA_{Test} of the sample into the equation to calculate the corresponding Karmen unit y value.

2. Calculation of AST Activity in Samples

Definition of Karmen unit: At 25°C, one Karmen unit is defined as the amount of pyruvate produced in 1 mL sample with a total reaction volume of 3 mL at a wavelength of 340 nm and 1 cm light path over 1 min, which causes a decrease of 0.001 in absorbance via oxidation of NADH to NAD⁺. (1 Karmen unit=0.482 U/L at 25°C)

Definition of international unit: One international unit (U) is defined as the quantity of enzyme that catalyzes the consumption of 1 μmol NADH per minute at 25°C in 1 L sample.

(1) Calculated by protein concentration

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

(2) Calculated by fresh weight of samples

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

(3) Calculated by bacteria or cell numbers

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

(4) Calculation of GOT activity in Serum (Plasma)

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

0.482: At 25 °C, 1 Karmen unit=0.482 U/L (international unit); $V_{\text{Sample total}}$: Volume of 1×PBS (pH 7.4) added for sample

pretreatment, 0.001 L; Cpr: sample protein concentration, g/L; W: sample weight, g; N: the number of cells, with the unit of 10^6 (for example, 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.

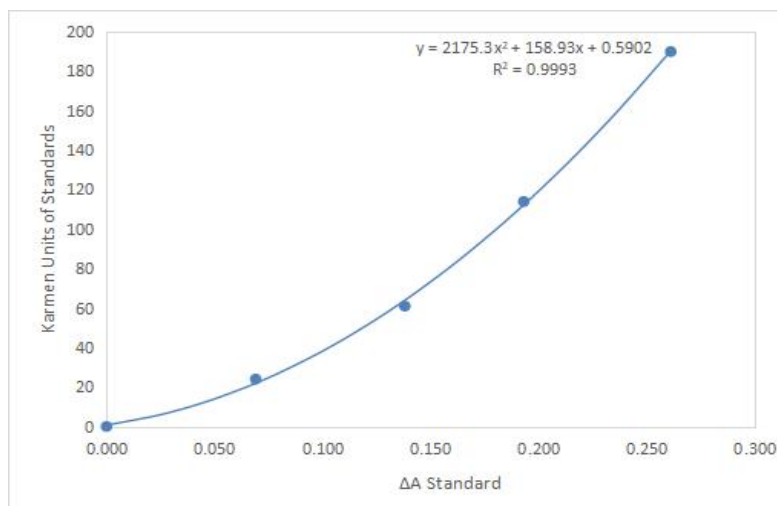


Figure 1. Standard curve for AST

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Alanine Aminotransferase (ALT/GPT) 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.