（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1301-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader(340 nm)

Detection range：5.81-1161.13 U/L

Elabscience® Aspartate Aminotransferase (AST/GOT) Activity Colorimetric Assay Kit (Aspartate Substrate Method)

This manual must be read attentively and completely before using this product. If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
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Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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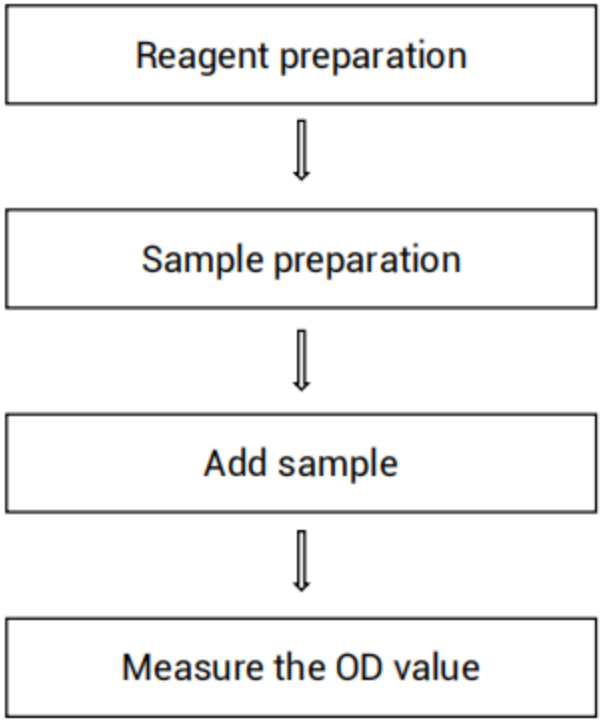
Assay summary



Intended use

This kit can be used to measure aspartate aminotransferase (AST/GOT) activity in serum (plasma), animal tissue and cell samples.

Detection principle

Glutamic-oxaloacetic transaminase (GOT), also known as aspartate aminotransferase (AST), is an important indicator reflecting liver function. AST in the sample catalyzes the deamination of L-aspartic acid to produce oxaloacetic acid. Oxaloacetic acid is reduced by the enzyme while NADH is oxidized to NAD^+, resulting in a decrease in the absorbance at 340 nm. By monitoring the rate of the decrease in absorbance at 340 nm, the enzymatic activity of AST can be determined.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Reagent	11 mL × 1 vial	22 mL × 1 vial	2-8°C, 12 months shading light
Reagent 2	Substrate Solution	2.8 mL × 1 vial	5.6 mL × 1 vial	2-8°C, 12 months shading light
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

Reagents:

PBS(0.01 M，pH 7.4)

Reagent preparation

Equilibrate all reagents to 25°C before use.

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL PBS(0.01 M，pH 7.4) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 8 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL PBS(0.01 M，pH 7.4) with a ultrasonic cell disruptor at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 8 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Human plasma	1
Mouse serum	1
10% Mouse liver tissue homogenate	20-50
10% Mouse kidney tissue homogenate	20-50
10% Mouse heart tissue homogenate	20-50
1×10^6 K562 cells	1
1×10^6 HL-60 cells	1

Note: The diluent is PBS(0.01 M，pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The ALT/GPT activity in human serum (plasma) samples is usually relatively low. The incubation time can be extended from 3-5 min when detecting such samples. Correspondingly, the reaction time in the calculation formula should be modified to 5 min.

② The A_1 value needs to be greater than 0.7 and the A_2 value needs to be greater than 0.2. To avoid enzyme activity exceeding the detection limit, the sample needs to be diluted if is not within this range.

Operating steps

① Sample well: add 10 μL of sample into sample wells.

② Add 200 μL of enzyme reagent into sample wells.

③ Mix fully and incubate at 37°C for 3 min.

④ Add 50 μL of substrate solution into sample wells.

⑤ Mix fully, measure the OD values of each well immediately at 340 nm with microplate reader, recorded as A_1.

⑥ Incubate at 37°C for 2 min, measure the OD value of each well immediately at 340 nm with microplate reader, recorded as A_2.

Calculation

The sample:

1. Serum or plasma samples:

Definition: The amount of 1 L serum or plasma per 1 min that consume 1 μmol of NADH at 37 °C is defined as 1 unit.

$$\underset{(U/L)}{\text{AST activity}} = \Delta A \div (\varepsilon \times d) \times \frac{V_2}{V_1} \times 10\text{^}6* \div T \times f = \Delta A \times 2903* \times f$$

2. Tissue or cell samples:

Definition: The amount of 1 g tissue or cell protein per 1 min that consume1 μmol of NADH at 37 °C is defined as 1 unit.

$$\underset{(U/gprot)}{\text{AST activity}} = \Delta A \div (\varepsilon \times d) \times \frac{V_2}{V_1} \times 10\text{^}6* \div T \div C_{pr} \times f = \Delta A \times 2903* \div C_{pr} \times f$$

3. Cell samples:

Definition: The amount of 1×10^6 cells per 1 min that consume 1 μmol of NADH at 37 °C is defined as 1 unit.

$$\underset{(U/10\text{^}6)}{\text{AST activity}} = \Delta A \times \frac{V_2}{\varepsilon \times d} \times 10\text{^}6^{*} \div T \div \left(n \times \frac{V_1}{V_3}\right) \times f = \Delta A \times 0.6^{**} \div n \times f$$

[Note]:

ΔA: $A_1 - A_2$.

ε: The molar extinction coefficient at 340 nm, 6.22×10^3 L/(mol·cm).

d: Optical path, 0.72 cm.

V_1: The volume of sample, 10 μL.

V_2: The volume of reaction system, 260 μL.

V_3: The volume of PBS(0.01 M，pH 7.4) added when homogenizing the cell sample, 200 μL = 2 × 10^-4 L.

10^6*:1 mol = 1×10^6 μmol.

C_{pr}: Concentration of protein in sample, gprot/L.

n: The amount of cell/1×10^6.

T: Reaction time, 2 min.

f: Dilution factor of sample before test.

2903*: Simplified value 1.

0.6**: Simplified value 2.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	71.0	142.2	292.2
%CV	4.8	2.6	2.9

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	31.0	66.4	135.5
%CV	3.4	5.8	2.1

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 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	73.1	146.1	292.2
Observed Conc. (U/L)	71.0	142.2	292.2
Recovery rate (%)	97	97	100

Sensitivity

The analytical sensitivity of the assay is 5.81 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

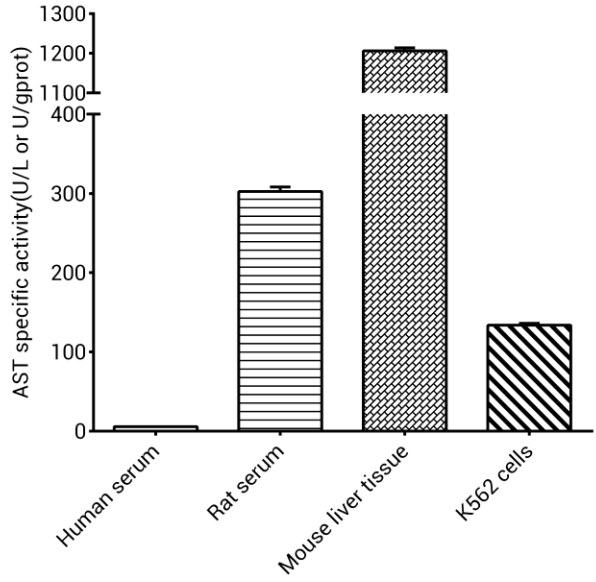
Example analysis：

Take 10 μL of rat serum and carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample well is 1.365, the A_2 of the sample well is 1.259, ΔA_{sample} = 1.365 - 1.259 = 0.106, and the calculation result is:

$$\text{AST activity (U/L)} = 0.106 \times 2903 = 307.72 \text{ U/L}$$

Detect human serum, rat serum, 10% mouse liver tissue homogenate (the concentration of protein is 10.83 gprot/L, dilute for 50 times), 1×10^6 K562 cells (the concentration of protein is 1.02 gprot/L) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.