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

Catalog No：E-BC-K236-S

Specification：100 Assays(45 samples)

Measuring instrument：Spectrophotometer（505 nm）

Detection range：0.38-72.30 IU/L

Elabscience® Alanine Aminotransferase (AST/GOT) Activity Assay Kit

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
Tell: 1-832-243-6086
Fax: 1-832-243-6017
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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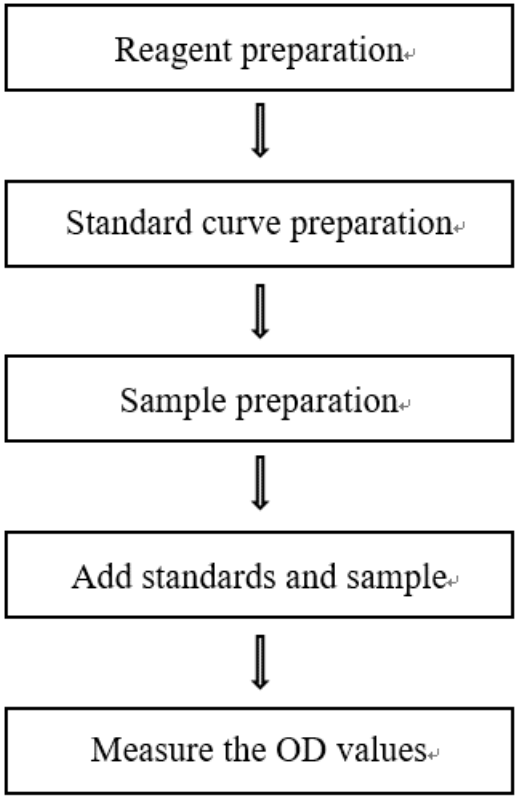
Assay summary

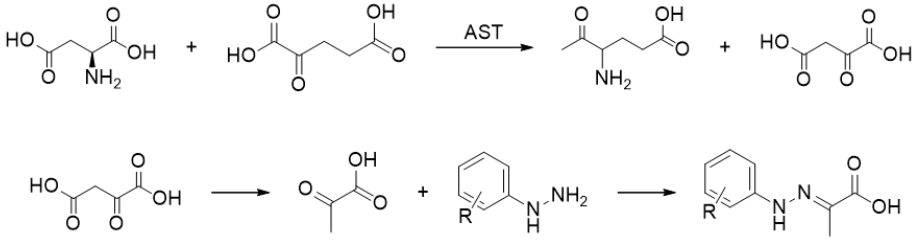


Intended use

This kit can be used to measure aspartate aminotransferase (AST/GOT) activity in serum (plasma), animal tissue, culture cells, etc.

Detection principle

Aspartate aminotransferase (AST) enables alpha-ketoglutaric acid and aspartic acid to displace amino to form glutamic acid and oxaloacetic acid. Oxaloacetic acid can decarboxylate itself to form Pyroracemic acid during the reaction. Pyroracemic acid reacted with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-dinitrophenylhydrazone showing reddish brown in alkaline solution. Measure the OD values and calculate the enzyme activity.



Kit components & storage

Item	Component	Size (100 assays)	Storage
Reagent 1	Buffer Solution	1.8 mL ×1 vial	2-8℃, 12 months
Reagent 2	2 mmol/L Sodium Pyruvate	1.8 mL ×1 vial	2-8℃, 12 months
Reagent 3	Substrate Solution	30 mL ×2 vials	2-8℃, 12 months
Reagent 4	Chromogenic Reagent	30 mL ×2 vials	2-8℃, 12 months shading light
Reagent 5	Alkali Reagent	30 mL ×2 vials	2-8℃, 12 months

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:

Test tube, Micropipettor, 37℃ Water bath, Vortex mixer, Spectrophotometry (505 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Incubate substrate solution at 37℃ for 10 min.

② The preparation of alkali working solution：

For each tube, prepare 5 mL of alkali working solution (mix well 0.5 mL of alkali reagent and 4.5 mL of double distilled water). The alkali working solution should be prepared on spot.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

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

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

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

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 300 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ 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 plasma	1
Human serum	1
10% Rat liver tissue homogenate	16-32
HepG2 supernatant	1
Rat serum	1
Mouse plasma	1
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① Add alkali working solution at the same rate.

② It is recommended to use fresh samples.(Serum samples can be stored at 2-8℃ for 1 week or -20℃ for 1 month.)

Operating steps

The measurement of standard curve

① Standard tubes: Record the test tube with A, B, C, D, E in duplication, add 0.1 mL of buffer solution to the standard tubes respectively. Add 0, 0.05, 0.10, 0.15, 0.20 mL of 2 mmol/L sodium pyruvate to the standard tubes from A to E, respectively. Add 0.50, 0.45, 0.40, 0.35, 0.30 mL of substrate solution to the standard tubes from A to E, respectively.

② Add 0.50 mL of chromogenic reagent to each tube.

③ Mix fully and incubate at 37℃ for 20 min.

④ Add 5 mL of alkali working solution to each tube.

⑤ Stand for 10 min at room temperature and set to zero with double distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm.

Note: Plot the standard curve by using absoluted OD value(OD value of each tube - OD value of A tube) of standard and correspondent concentration (0, 24, 61, 114, 190) as x-axis and y-axis respectively. Create the standard curve (**$y = ax^2 + bx + c$**) with graph software (or EXCEL).

The measurement of samples

① Sample tubes: add 0.1 mL of sample to 10 mL EP tube, then add 0.5 mL of substrate solution (pre-heated at 37℃ for 10 min) to 10 mL EP tube.
Control tubes: add 0.5 mL of substrate solution (pre-heated at 37℃ for 10 min) to 10 mL EP tube.

② Mix well and incubate at 37℃ for 30 min.

③ Add 0.50 mL of chromogenic reagent to each tube.

④ Control tubes: Add 0.1 mL of sample to control tubes.

⑤ Mix well and incubate at 37℃ for 20 min.

⑥ Add 5 mL of alkali working solution to each tube.

⑦ Stand for 10 min at room temperature and set to zero with double distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #A) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration (0, 24, 61, 114, 190) as x-axis and y-axis respectively. Create the standard curve (**$y = ax^2 + bx + c$**) with graph software (or EXCEL).

Definition of international unit: The enzyme amount of 1 μmol of NADH consumed in reaction system 25 ℃ per minute is defined as 1 unit.

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 cause absorbance decreasing 0.001 is as 1 unit. (1 carmen unit = 0.482 IU/L, 25 ℃).

The sample:

1. Serum (plasma) sample:

$$\text{AST activity (IU/L)} = [a \times (\Delta A_{505})^2 + b \times \Delta A_{510} + c] \times 0.482 \times f$$

2. Tissue and cells sample:

$$\text{AST activity (IU/gprot)} = [a \times (\Delta A_{505})^2 + b \times \Delta A_{510} + c] \times 0.482 \times f \div C_{pr}$$

[Note]

ΔA_{505}: OD_{sample} - $OD_{control}$

f: Dilution factor of sample before tested.

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

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 (IU/L)	5.50	26.50	54.80
%CV	4.7	4.1	3.8

Inter-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 (IU/L)	5.50	26.50	54.80
%CV	4.8	5.2	4.7

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 104%.

	Standard 1	Standard 2	Standard 3
Expected Conc. （Carmen unit）	24	61	114
Observed Conc. （Carmen unit）	25.4	62.8	117.4
recovery rate(%)	106	103	103

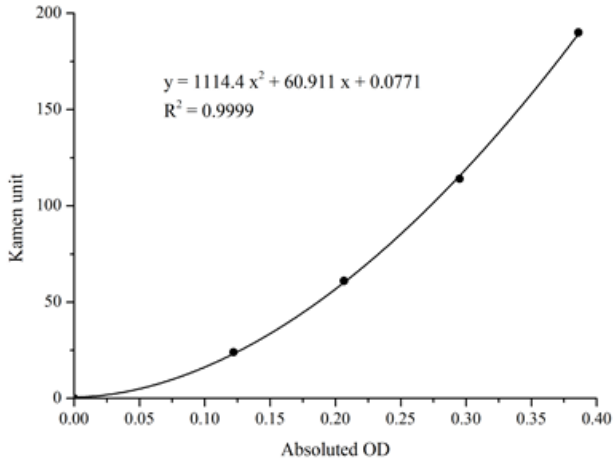
Sensitivity

The analytical sensitivity of the assay is 0.38 IU/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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Carmen unit	0	24	61	114	190
Average OD	0.222	0.344	0.429	0.517	0.608
Absoluted OD	0.000	0.122	0.207	0.295	0.386



Appendix II Example Analysis

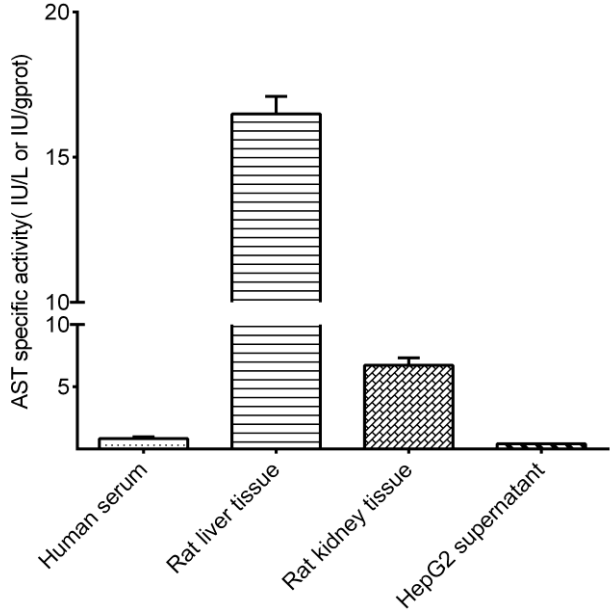
Example analysis:

For human serum, take 0.1 mL of human serum and carry the assay according to the operation steps. The results are as follows:

standard curve: $y = 1114.4\ x^2 + 60.911\ x + 0.0771$, the average OD value of the sample is 0.271, the average OD value of the control is 0.251, and the calculation result is:

$$\text{AST activity (IU/L)} = [1114.4 \times (0.271 - 0.251)^2 + 60.911 \times (0.271 - 0.251) + 0.0771] \times 0.482 = 0.84\ \text{IU/L}$$

Detect human serum, 10% rat liver tissue homogenate (the concentration of protein is 13.11 gprot/L dilute for 50 times), 10% rat kidney tissue homogenate (the concentration of protein is 9.26 gprot/L) and HepG2 supernatant 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.