PRODUCT INFORMATION & MANUAL

Aspartate Aminotransferase Activity Assay Kit (Colorimetric)

NBP3-24470

For research use only.
Not for diagnostic or therapeutic procedures.

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Aspartate Aminotransferase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24470

Method: Colorimetric method

Specification: 100Assays (Can detect 45 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.38 IU/L

Detection range: 0.38-72.30 IU/L

Average intra-assay CV (%): 4.2

Average inter-assay CV (%): 4.9

Average recovery rate (%): 104

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

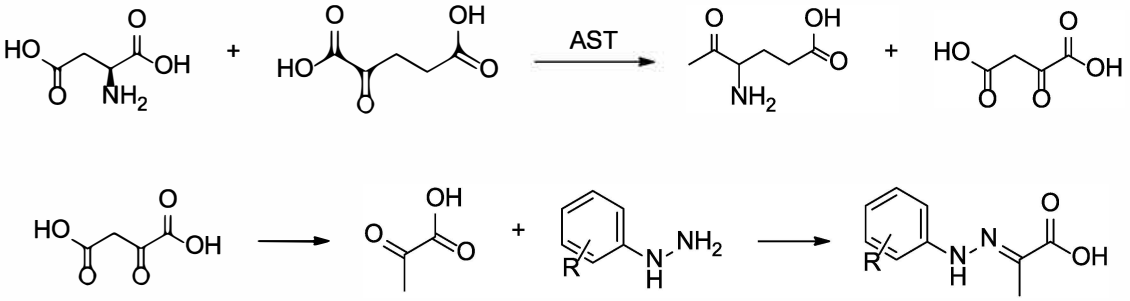
This kit can be used to measure aspartate aminotransferase (AST/GOT) activity in serum (plasma), animal tissue, culture cells, etc.

▲Background

Aspartate aminotransferase (AST) is a key enzyme in nitrogen metabolism, which is widely found in plasma and body tissues, including liver, heart, skeletal muscle, kidney, brain, pancreas, lung and erythrocyte. Changes in AST activity were found in acute pancreatitis, ischemic stroke, severe burns, periodontitis, acute renal disease and motor neuron disease.

▲Detection principle

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	Specification	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.			

▲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)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. Add reagent 5 working solution at the same rate.
2. Serum samples can be stored at 2-8℃ for 1 week or -20℃ for 1 month.

Pre-assay preparation

▲Reagent preparation

Preparation of reagent 5 working solution:

Dilute the reagent 5 with double distilled water at the ratio of 1: 9 and mix fully. Prepare the fresh solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.38-72.30 IU/L).

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).

Assay protocol

▲Detailed operating steps

The preparation of standard curve

1) Standard tubes: Record the test tube with A, B, C, D, E in duplication, add 0.1 mL of regent 1 to the standard tubes respectively. Add 0, 0.05, 0.10, 0.15, 0.20 mL of reagent 2 to the standard tubes from A to E, respectively. Add 0.50, 0.45, 0.40, 0.35, 0.30 mL of reagent 3 to the standard tubes from A to E, respectively.
2) Add 0.50 mL of reagent 4 to each well.
3) Mix fully and incubate at 37℃ for 20 min.
4) Add 5 mL of reagent 5 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.

The measurement of samples

1) Sample tubes: Add 0.1 mL of sample to 10 mL EP tub, then add 0.5 mL of reagent 3 (pre-heated at 37℃ for 10 min) to 10 mL EP tube.

   Control tubes: Add 0.5 mL of reagent 3 (pre-heated at 37℃ for 10 min) to 10 mL EP tube.
2) Mix fully and incubate at 37℃ for 30 min.
3) Add 0.5 mL of reagent 4 to each tube.
4) Sample tubes: Add nothing.
   Control tubes: Add 0.1 mL of sample to control tubes.

5) Mix fully and incubate at 37℃ for 20 min.

6) Add 5 mL of reagent 5 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.

▲Summary operation table

1. The preparation of standard curve

	A	B	C	D	E
Reagent 1 (mL)	0.10	0.10	0.10	0.10	0.10
Reagent 2 (mL)	0	0.05	0.10	0.15	0.20
Reagent 3 (mL)	0.50	0.45	0.40	0.35	0.30
Reagent 4 (mL)	0.50	0.50	0.50	0.50	0.50
Mix fully, then incubate at 37℃ for 20 min.					
Reagent 5 working solution (mL)	5	5	5	5	5
Mix fully and stand for 10 min at room temperature. Set spectrophotometer to zero with double-distilled water and measure the OD value at 505 nm.					

2. The measurement of samples

	Control tube	Sample tube
Sample (mL)		0.1
Reagent 3 (mL) (pre-heated at 37℃ for 10 min)	0.5	0.5
Mix fully, then incubate at 37℃ for 30 min.		
Reagent 4 (mL)	0.5	0.5
Sample (mL)	0.1	
Mix fully and incubate at 37℃ for 20 min.		
Reagent 5 working solution (mL)	5	5
Mix fully and stand for 10 min at room temperature. Set spectrophotometer to zero with double-distilled water and measure the OD value at 505 nm.		

Note: Steps 1 and 2 can be progress at the same time.

▲Calculation

1. Definition of international unit: The enzyme amount of 1 μmol of NADH consumed in reaction system 25℃ per minute is defined as 1 unit .
2. Definition of carman 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 carman unit = 0.482 IU/L, 25℃).

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: $y=ax^2 + bx + c$.

1.Serum (plasma) sample:

$$\text{AST activity (IU/L)}=[a\times(OD_{sample}-OD_{control})^2+b(OD_{sample}-OD_{control})+c]\times 0.482\ \text{IU/L}\times f$$

2. Tissue and cell sample:

$$\text{AST activity (IU/gprot)}=[a\times(OD_{sample}-OD_{control})^2+b(OD_{sample}-OD_{control})+c]\times 0.482\ \text{IU/L}\times f\div C_{pr}$$

Note:

y: carman 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.

f: dilution factor of sample before tested.

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

Appendix I Data

▲Example analysis

For human serum, take 0.1 mL of human serum and carry the assay according to the operation table. 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:

AST activity (IU/L)=$[1114.4\times(0.271-0.251)^2+60.911\times(0.271-0.251)+0.0771]\times0.482$

=0.84 IU/L

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice 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 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲ Cells

Collect the cells and wash the cells with homogenization medium for 1~2 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): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) including 0.1 mM EDTA.
2. Homogenized method:
   (1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

   Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

   (2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

   (3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

Appendix II References

1. Karmen A, Wroblewski F, Ladue J S. Transaminase activity in human blood. Journal of Clinical Investigation, 1995, 34(1): 126-131.
2. Desai S N, Desai P V. Aspartate aminotransferase and alanine aminotransferase activities of rat brain during crush syndrome. Neuroscience Letters, 2008, 447(1): 58-61.
3. Campos F, Sobrino T, Ramoscabrer P, et al. Neuroprotection by glutamate oxaloacetate transaminase in ischemic stroke: an experimental study. J Cereb Blood Flow Metab, 2011, 31(6): 1378-1386.
4. Hugon J, Tabaraud F, Rigaud M, et al. Glutamate dehydrogenase and aspartate aminotransferase in leukocytes of patients with motor neuron disease. Neurology, 1989, 39(7): 956-958.