Aspartate Aminotransferase (AST/GOT) Activity Assay Kit

Catalog Number EEA003 (96 tests)

Rev 1.0

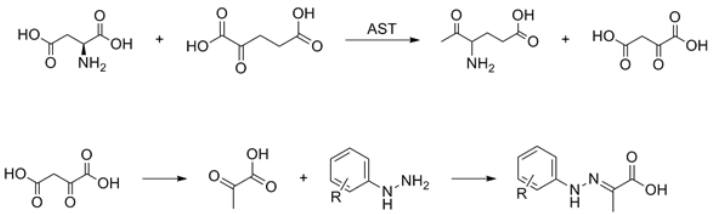
For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can measure Aspartate Aminotransferase (AST/GOT) activity in animal serum, plasma, tissue, cultured cells, and cell culture supernatant.

AST/GOT 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 erythrocytes. Changes in AST/GOT activity were found in acute pancreatitis, ischemic stroke, severe burns, periodontitis, acute renal disease, and motor neuron disease.

AST/GOT enables alpha-ketoglutaric acid and aspartic acid to displace amino and keto groups to form glutamic acid and oxaloacetic acid. Oxaloacetic acid can decarboxylate itself to form pyroracemic acid during the reaction. Pyroracemic acid reacts with 2,4-dinitro phenyl hydrazine (DNPH) to form 2,4, dinitrophenylhydrazone, showing reddish-brown color in alkaline solution. The OD values can be measured to calculate the enzyme activity.



Contents and storage

Kit and components are shipped at 2-8 ℃. An unopened kit can be stored at 2-8 ℃ for 12 months.

Components	Quantity (96 tests)
Buffer Solution	0.5 mL
2 mmol/L Sodium Pyruvate	0.5 mL
Substrate Solution	5 mL
Chromogenic Agent	5 mL
Alkali Reagent	5 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 510 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37 ℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant 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 0.02-1 g fresh tissue to wash with homogenization medium at 2-8 ℃ to remove blood cells.
- 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 and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80 ℃ for 1 month

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): PBS (0.01 M, pH 7.4) (μL) =1: 300-500.
- Sonicate or grind with hand-operated in ice water bath.
- Centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the cells sample (without homogenization) can be stored at -80 °C for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.1-72.3 IU/L).

Note: Use all samples within 2 hours of dilution

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

Sample type	Dilution factor
Human serum	1
Human plasma	1
Porcine serum	1
Rat serum	1
HC-60 cellular supernatant	1
Calu-3 cellular supernatant	1
10% Rat liver tissue homogenization	15-30
10% Rat lung tissue homogenization	2-8

Note: The diluent is normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4).

Preparation of alkali reagent working solution

Mixing 1 parts of the alkali reagent with 9 part of distilled water at the ratio of 1: 9 and mix fully. Prepare the fresh solution before use.

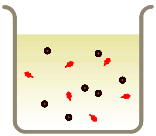
Preparation of substrate solution

Incubate at 37 ℃ for 10 min before use.

Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Assay procedure



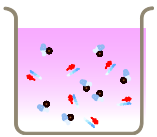
1. Add sample, standard and control

a. **Standard well:** Add 5 μL of buffer solution to the standard wells. Add 20, 18, 16, 14, 12 μL of substrate solution to the standard wells from A to E, respectively. Add 0, 2, 4, 6, 8 μL of 2 mmol/L sodium pyruvate to the standard wells from A to E, respectively.

b. **Sample well:** Add 20 μL of substrate solution (pre-heated at 37 ℃ for 10 min) and 5 μL of sample.

Control well: Add 20 μL of substrate solution (pre-heated at 37 ℃ for 10 min).

c. Mix fully and incubate at 37 ℃ for 30 min.



2. Add substrate

a. Add 20 μL of chromogenic agent to each well.

b. Add 5 μL of sample to Control wells.

c. Mix fully with microplate reader for 10 s, incubate at 37 ℃ for 20 min.

d. Add 200 μL of alkali reagent working solution to each well (a multi-channel pipette is recommended).

e. Mix fully with microplate reader for 10 s, then let stand for 15 min at room temperature and measure the OD value of each well with a microplate reader at 510 nm.

Calculation

1. **Definition of international unit**: The enzyme amount of 1 μmol of NADH consumed in reaction system (1 mL sample or 1 g tissue protein, 25 ℃) per minute is defined as 1 unit (wavelength is 340 nm, optical path is 1 cm).

2. **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 ℃).

3. Plot the standard curve by using OD value of standard and correspondent Carmen unit (0, 28, 57, 97, 150, 200 Carmen unit) as x-axis and y-axis respectively. Create the standard curve with graph software (or EXCEL). The Carmen unit of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is $y=ax^2+bx+c$.

Serum (plasma) and other liquid sample:

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

Tissue and cells sample:

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

[Note]

y: Carmen 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

ΔA_{510}: $OD_{sample} - OD_{control}$

f: dilution factor of sample before tested

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

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

Take 5 μL of human serum, carry the assay according to the operation table. The results are as follows:

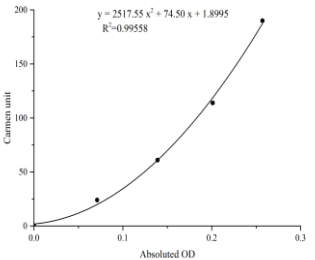
standard curve: y = 2517.55 x2 + 74.50 x + 1.8995, the average OD value of the sample is 0.259, the average OD value of the control is 0.233, and the calculation result is:

$$\text{AST activity (IU/L)} = [2517.55 \times (0.259 - 0.233)^2 + 74.50 \times (0.259 - 0.233) + 1.8995] \times 0.482 = 2.67 \text{ IU/L}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 1.1-72.3 IU/L standard.

Concentration (Carmen unit)	OD at 510 nm	Standard Curve
190.00	0.479	
114.00	0.423	
61.00	0.361	
24.00	0.293	
0.00	0.222	

- Inter-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (Carmen unit)	24.00	61.00	114.00
%CV	7.9	6.4	6.1

CV = Coefficient of Variation

- Intra-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 (Carmen unit)	24.00	61.00	114.00
%CV	5.5	5.0	5.4

CV = Coefficient of Variation

- Expected values

This assay was tested with serum samples without dilutions.

Sample Type	Range (**IU/L**)	Average (**IU/L**)
Serum	15-40	27.5

▪ 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 97%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (Carmen unit)	24	61	114
Observed Conc. (Carmen unit)	26.04	59.85	153
Recovery rate (%)	96	97	99

▪ Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
B	B	B	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
C	C	C	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
D	D	D	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
E	E	E	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'
F	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
G	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
H	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'

[Note]: A-E, standard wells; S1-S43, sample wells; S1'-S43, control wells.

- Sensitivity

The analytical sensitivity of the assay is 1.10 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.

Limited product warranty

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June 2023

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