Technical Manual

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

- Catalogue Code: MAES0158
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

1.1-72.3 IU/L

Sensitivity:

1.1 IU/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

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

3. Intended Use

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

4. Detection Principle

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 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.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	0.5 mL × 1 vial	2-8°C, 6 months
Sodium Pyruvate (2 mmol/L)	0.5 mL × 1 vial	2-8°C, 6 months
Substrate Solution	5 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent	5 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Alkali Reagent	5 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Incubator
- Multichannel pipette
- Microplate Reader (500-520 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. It is recommended to use a multi-channel pipette to add alkali working solution to reduce the difference between wells.

2. Detect the sample as soon as possible after collection. The serum sample can be store at 2-8°C for 7 days and -20°C for 20 days.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **alkali working solution**: Dilute the alkali reagent with double distilled water at the ratio of 1: 9 and mix fully. Prepare the fresh solution before use.

3. Incubate substrate solution at 37°C for 10 min.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) 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 (2×10^6): PBS (0.01 M, pH 7.4) including 0.1 mM EDTA (μL) =1: 400. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) including EDTA (0.1 mM) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (1.1-72.3 IU/L).

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) or PBS (0.01 M, pH 7.4).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 510 nm

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', control wells; S1-S43, sample wells.

10. Operation Steps

1. **Standard wells:** Add 5 μL of buffer solution to the standard wells respectively (the pipettes should touch the bottom of the plate). 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.
   Sample wells: Add 20 μL of substrate solution (pre-heated at 37°C for 10 min) and 5 μL of sample.
   Control wells: Add 20 μL of substrate solution (pre-heated at 37°C for 10 min).

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

3. Add 5 μL of sample to **control wells**.

4. Mix fully with microplate reader for 10s, incubate at 37°C for 20 min.

5. Add 200 μL of alkali working solution to each well (the multi-channel pipette is recommended).

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

Operation Table

The preparation of standard curve

Set 5 wells of micro-plate for standard and operate according to the following operating table.

	A	B	C	D	E
Buffer solution (μL)	5	5	5	5	5
Substrate solution (μL)	20	18	16	14	12
Sodium pyruvate (2 mmol/L) (μL)	0	2	4	6	8
Mix fully (this is very important), then incubate at 37°C for 30 min.					
Chromogenic agent (μL)	20	20	20	20	20
Mix fully with microplate reader for 10 s and incubate at 37°C for 20 min.					
Alkali working solution (μL)	200	200	200	200	200
Mix fully with microplate reader for 10 s, stand for 15 min at room temperature and measure the OD value of each well with microplate reader at 510 nm.					

The measurement of samples

	Control well	Sample well
Substrate solution (μL) (pre-heated at 37°C for 10 min)	20	20
Sample (μL)		5
Mix fully (this is very important), then incubate at 37°C for 30 min		
Chromogenic agent (μL)	20	20
Sample (μL)	5	
Mix fully with microplate reader for 10 s and incubate at 37°C for 20 min		
Alkali working solution (μL)	200	200
Mix fully with microplate reader for 10 s, stand for 15 min at room temperature and measure the OD value of each well with microplate reader at 510 nm		

11. Calculations

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°C) 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°C 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°C**).

3. Plot the standard curve by using OD value of standard and correspondent Carmen unit (0, 24, 61, 114, 190 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 $y=ax^2+bx+c$.

1. Serum (plasma) sample:

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

2. Tissue and Cells sample:

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

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

12. Performance Characteristic

Detection Range	1.1-72.3 IU/L
Sensitivity	1.1 IU/L
Average inter-assay CV (%)	6.8
Average intra-assay CV (%)	5.3

Analysis

Take 5 μL of human serum, carry the assay according to the operation table.

The results are as follows:

standard curve: $y = 2517.55\ x^2 + 74.50\ x + 1.8995$, 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:

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

$$= 2.67\ \text{IU/}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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Notes:

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