TECHNICAL MANUAL

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

- **SKU CODE:** MAES0158
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

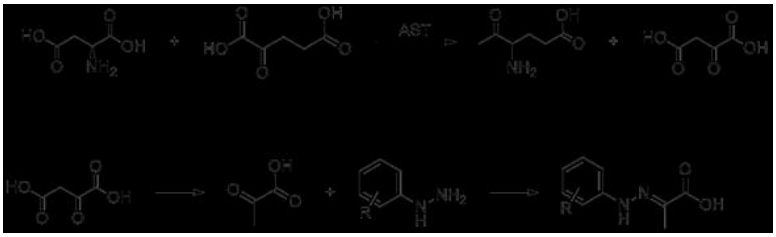
- Reagent preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 30 min
- Add chromogenic agent
- Add samples to control wells
- Incubate at 37°C for 20 min
- Add alkali working solution
- Measure absorbance at 510 nm

2. Intended use

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

3. Detection principle

AST/GOT catalyzes the transfer of amino groups between alpha-ketoglutaric acid and aspartic acid to form glutamic acid and oxaloacetic acid. Oxaloacetic acid undergoes decarboxylation to form pyruvic acid during the reaction. Pyruvic acid reacts with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-dinitrophenylhydrazone, which shows a reddish-brown color in alkaline solution. The optical density values are measured and used to calculate enzyme activity.



4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Buffer Solution	0.5 mL × 1 vial	0.5 mL × 1 vial	2.5 mL × 1 vial	2-8°C, 12 months
Reagent 2	2 mmol/L Sodium Pyruvate	0.5 mL × 1 vial	0.5 mL × 1 vial	2.5 mL × 1 vial	2-8°C, 12 months
Reagent 3	Substrate Solution	2.5 mL × 1 vial	5 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent	2.5 mL × 1 vial	5 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months, protect from light
Reagent 5	Alkali Reagent	2.5 mL × 1 vial	5 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (500-520 nm), Vortex mixer, Incubator, Multichannel pipette

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. **Preparation of alkali working solution:** For each well, prepare 200 µL of alkali working solution by mixing 20 µL of alkali reagent with 180 µL of double distilled water. The alkali working solution should be prepared immediately before use.
2. Incubate substrate solution at 37°C for 10 minutes before use.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one month.

Urine: Collect fresh urine and centrifuge at 10,000 × g for 15 minutes at 4°C. Take the supernatant and preserve it on ice for detection.

Tissue sample:

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

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

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

4. Centrifuge at 10,000 × 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 (MAES0177).

Cells:

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

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1×10^6 cells in 300 µL PBS (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000 × 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 (MAES0177).

Sample dilution:

The recommended dilution factors for different samples are as follows (for reference only):

- Human serum: 1
- Human plasma: 1
- Porcine serum: 1
- Rat serum: 1
- HC-60 cellular supernatant: 1
- Calu-3 cellular supernatant: 1
- 10% Mouse liver tissue homogenate: 15-30
- 10% Mouse lung tissue homogenate: 2-8

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

- It is recommended to use a multichannel pipette to add alkali working solution to reduce variation between wells.
- Detect samples as soon as possible after collection. Serum samples can be stored at 2-8°C for 7 days and at -20°C for 20 days.

9. Operating steps

1. **Standard wells:** Add 5 μL of buffer solution to the standard wells (pipette tips should touch the bottom of the plate). Add 20, 18, 16, 14, 12 μL of substrate solution to standard wells A to E, respectively. Add 0, 2, 4, 6, 8 μL of reagent 2 to standard wells A to E, respectively. Sample wells: Add 20 μL of substrate solution (pre-heated at 37°C for 10 minutes) and 5 μL of sample. Control wells: Add 20 μL of substrate solution (pre-heated at 37°C for 10 minutes).
2. Mix well (this is very important), then incubate at 37°C for 30 minutes.
3. Add 20 μL of chromogenic agent to each well.
4. Control wells: Add 5 μL of sample to control wells.
5. Mix well with microplate reader for 10 seconds, incubate at 37°C for 20 minutes.
6. Add 200 μL of alkali working solution to each well (multichannel pipette is recommended).
7. Mix thoroughly with microplate reader for 10 seconds, stand for 15 minutes at room temperature and measure the OD value of each well with microplate reader at 510 nm.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using absolute OD values of standards and corresponding concentrations (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 that consumes 1 μmol of NADH in the reaction system (1 mL sample/1 g tissue protein, 25°C) per minute is defined as 1 unit (wavelength is 340 nm, optical path is 1 cm).

Definition of Carmen unit: 1 mL of sample, total reaction volume of 3 mL, wavelength of 340 nm, optical path of 1 cm, reacted at 25°C for 1 minute, the amount of generated pyruvic acid that oxidizes NADH to NAD+ and causes absorbance to decrease by 0.001 is defined as 1 unit. (1 Carmen unit = 0.482 IU/L, 25°C).

The sample:

1. Serum (plasma) sample:

AST/GOT activity (IU/L) = $[a \times (\Delta A510)^2 + b \times \Delta A510 + c] \times 0.482 \times f$

2. Tissue and cells sample:

AST/GOT activity (IU/gprot) = $[a \times (\Delta A510)^2 + b \times \Delta A510 + c] \times 0.482 \times f \div C_{pr}$

[Note]

ΔA510: $OD_{sample} - OD_{control}$

f: Dilution factor of sample before testing

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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Recovery

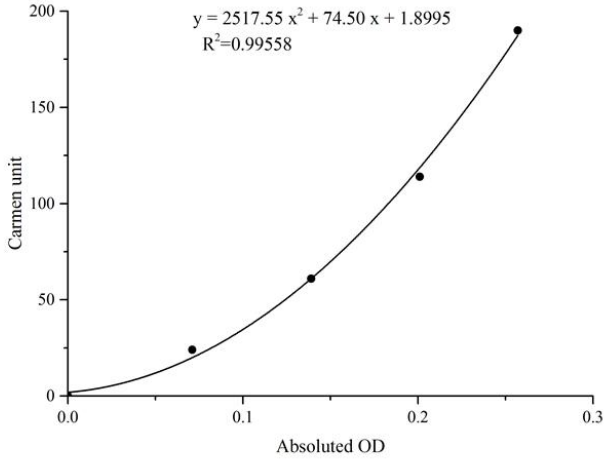
Three samples of high, middle, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 97%.

Sensitivity

The analytical sensitivity of the assay is 1.1 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.

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), the standard curve and data are provided below for reference only:



12. Appendix II Example Analysis

Example analysis:

Take 5 μL of human serum and perform the assay according to the operating steps. The results are as follows:

Standard curve: $y = 2517.55x^2 + 74.50x + 1.8995$

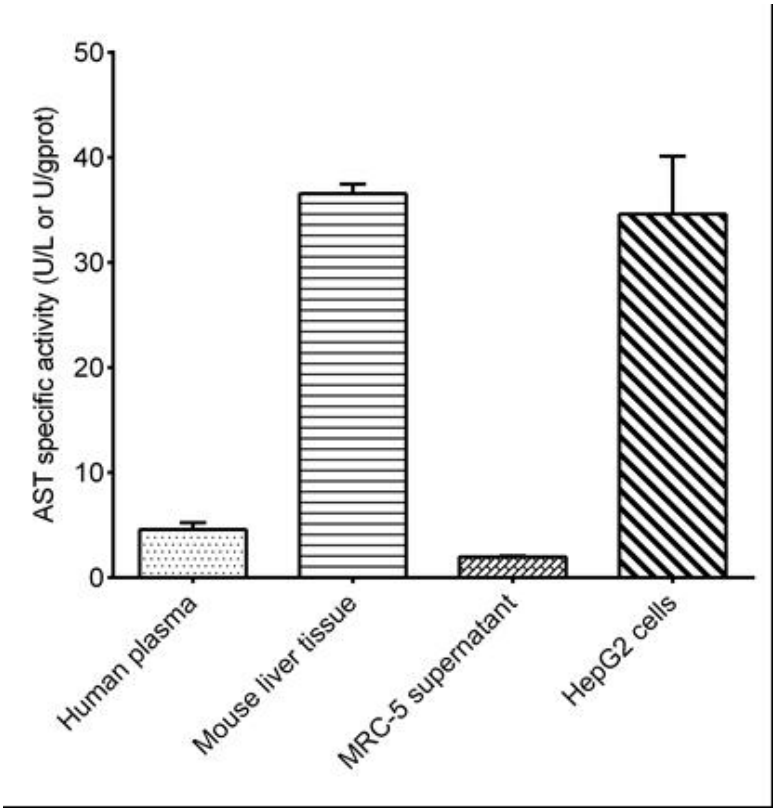
Average OD value of sample: 0.259

Average OD value of control: 0.233

Calculation result:

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 IU/L

Detect human plasma, 10% mouse liver tissue homogenate (protein concentration: 9.58 gprot/L, diluted 20 times), culture supernatant of MRC-5 cells and HepG2 cells (protein concentration: 3.39 gprot/L, diluted 5 times) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing experiments. Follow the instructions strictly during the experiments.

3. Protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance is not within the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to calculate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.