TECHNICAL MANUAL

AST Fluorometric Activity Assay Kit

- **SKU CODE:** MAES0253
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Standard curve preparation
- Add standards and samples
- Measure fluorescence values
- Calculate AST activity

2. Intended use

This kit can be used to measure aspartate aminotransferase (AST/GOT) activity in cell, animal tissue, serum (plasma) and other liquid samples.

3. Detection principle

Aspartate aminotransferase (AST/GOT) is an important indicator of liver inflammation. When cells are damaged, the permeability of cell membrane increases, resulting in the release of AST from the cytoplasm into the blood and an increase in serum AST activity. AST catalyzes the substrate to produce pyruvate. Pyruvate is oxidized to produce hydrogen peroxide, which causes the fluorescent probe to fluoresce. The activity of AST can be calculated by measuring the increase in fluorescence value at an excitation wavelength of 535 nm and an emission wavelength of 587 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 2 vials	-20°C, 12 months
Reagent 2	Substrate	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Aqueous Alkali	0.3 mL × 1 vial	0.6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	100 mmol/L Pyruvate Standard	1 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Micropipette, Vortex mixer, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/587 nm)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution::** Dissolve one vial of enzyme reagent with 1.2 mL of buffer solution, mix well to dissolve completely. Keep enzyme working solution on ice protected from light during use. Store at -20°C for 7 days protected from light, and avoid repeated freeze/thaw cycles.
3. **Preparation of reaction working solution::** For each well, prepare 100 µL of reaction working solution (mix well 50 µL of buffer solution, 25 µL of substrate, 20 µL of enzyme working solution and 5 µL of aqueous alkali). Keep reaction working solution on ice protected from light during use. The reaction working solution should be prepared fresh and the prepared solution should be used within 1 hour.
4. **Preparation of 1 mmol/L pyruvic acid standard stock solution::** Dilute 10 µL of 100 mmol/L pyruvate standard with 990 µL of buffer solution, mix well to dissolve. The 1 mmol/L pyruvic acid standard stock solution should be prepared fresh.
5. **Preparation of 100 µmol/L pyruvic acid standard solution::** Dilute 1 mmol/L pyruvic acid standard stock solution with 990 µL of buffer solution, mix well to dissolve. The 100 µmol/L pyruvic acid standard solution should be prepared fresh.

7. Standard curve preparation

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 µmol/L pyruvic acid standard solution with buffer solution to create a serial concentration. The recommended dilution gradient is as follows: 80, 60, 50, 40, 30, 20, 10, 0 µmol/L.

Standard preparation table:

Item ① ② ③ ④ ⑤ ⑥ ⑦ ⑧

Concentration (μmol/L): 0, 10, 20, 30, 40, 50, 60, 80

100 μmol/L pyruvic acid standard (μL): 0, 20, 40, 60, 80, 100, 120, 160

Buffer solution (μL): 200, 180, 160, 140, 120, 100, 80, 40

8. Sample preparation

1. **Serum and plasma::** Detect the sample directly. If the sample contains lipids, chylous material, etc., centrifuge at 5000×g at 4°C for 5 min, collect the clarified supernatant and store on ice for testing.
2. **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 buffer solution with a dounce homogenizer at 4°C. 4) Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection. 5) Meanwhile, determine the protein concentration of supernatant (MAES0177).
3. **Cell (adherent or suspension) samples::** 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 1 mL buffer solution with an ultrasonic cell disruptor at 4°C. 4) Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection. 5) Meanwhile, determine the protein concentration of supernatant (MAES0177).

9. Sample dilution

Sample type	Dilution factor
10% Rat heart tissue homogenate	300-600
10% Rat brain tissue homogenate	300-500
10% Rat liver tissue homogenate	200-500
Human serum	5-15
10% Rat kidney tissue homogenate	200-500
Rat serum	10-20
10% Rat spleen tissue homogenate	50-100

Sample type	Dilution factor
293T cell(1×10^6)	10-20
10% Mouse lung tissue homogenate	100-200
293T cell supernatant	5-15

10. The key points of the assay

Prevent the formation of bubbles when transferring the supernatant into the microplate.

11. Operating steps

1. Standard well: Add 20 μL of standard with different concentrations into the corresponding wells. Sample well: Add 20 μL of sample into the corresponding wells.
2. Add 100 μL of reaction working solution to each well.
3. Mix thoroughly with microplate reader for 5 s and stand at room temperature for 3 min.
4. Measure the fluorescence intensity of each well at an excitation wavelength of 535 nm and an emission wavelength of 587 nm, recorded as F1, then react at room temperature for 60 min with shading light. Determine the fluorescence intensity of each well under the same wavelength, and record as F2, then ΔF = F2-F1 (Note: There is no change in fluorescence value of standard wells, plot the standard curve with the fluorescence value of F2(standard)).

12. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard ①) from all standard readings. This is the absolute fluorescence value.

3. Plot the standard curve using absolute fluorescence value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve ($y = a_1x + b_1$) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of enzyme in 1 L of serum or plasma that catalyzes the production of 1 μmol pyruvic acid per minute at 25°C is defined as 1 unit.

AST activity (U/L) = (ΔF-b)/a ÷ T × f

2. Tissue and cell samples:

Definition: The amount of enzyme in 1 g of sample protein that catalyzes the production of 1 μmol pyruvic acid per minute at 25°C is defined as 1 unit.

AST activity (U/gprot) = (ΔF-b)/a ÷ T × f ÷ C_{pr}

[Note]

ΔF: The absolute fluorescence value of sample, ΔF = F_2 - F_1.

T: The reaction time, 60 min.

f: Dilution factor of sample before tested.

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

13. 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)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.10	0.80	1.20
%CV	2.5	2.2	1.9

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.10	0.80	1.20
%CV	6.7	7.5	7.7

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with 6 replicates for each concentration to obtain an average recovery rate of 100%.

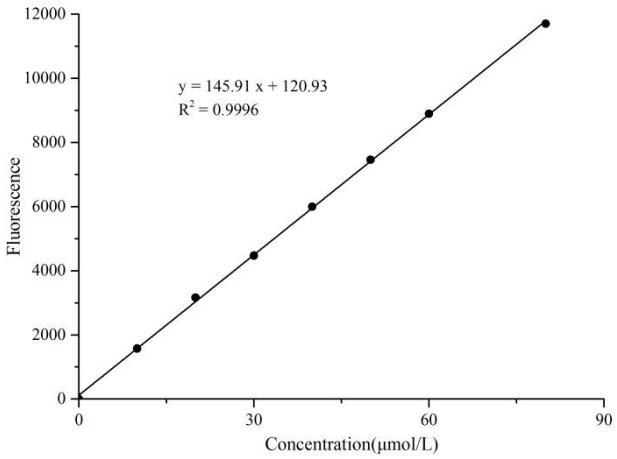
	Standard 1	Standard 2	Standard 3
Expected Conc.(µmol/L)	15	36.5	55
Observed Conc.(µmol/L)	14.9	35.8	56.7
Recovery rate (%)	99	98	103

Sensitivity

The analytical sensitivity of the assay is 0.03 U/L. This was determined by adding two standard deviations to the mean fluorescence value obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the fluorescence 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:



Concentration (μmol/L)	Average fluorescence value	Absolute fluorescence value
0	1019	0
10	2598	1579
20	4185	3166
30	5490	4471
40	7018	5999
50	8482	7463
60	9917	8898
80	12725	11706

14. Appendix II Example Analysis

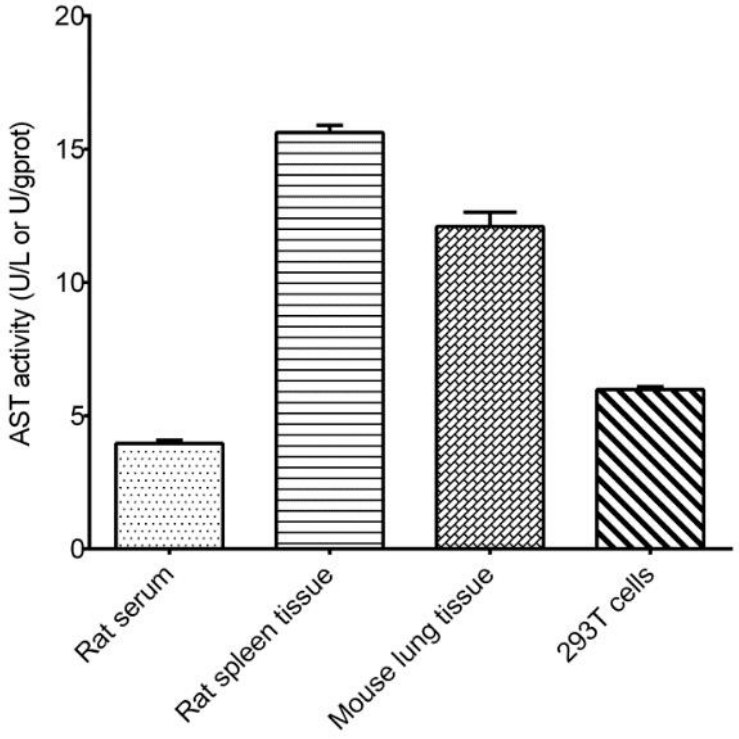
Example analysis:

For 10% rat spleen tissue, diluted 80-fold, 20 μL was used for detection, and the assay was carried out according to the operating procedure. The results are as follows:

Standard curve: y = 96.281x + 202.56, the average fluorescence value of the sample F_1 is 1163, the average fluorescence value of the sample F_2 is 9155, the concentration of protein in sample is 6.90 gprot/L, and the calculation result is:

AST activity (U/gprot) = (9155 - 1163 - 202.56) ÷ 96.281 ÷ 60 × 80 ÷ 6.90 = 15.63 U/gprot

Detection of rat serum (diluted 10-fold), 10% rat spleen tissue homogenate (protein concentration 6.90 gprot/L, diluted 80-fold), 10% mouse lung tissue homogenate (protein concentration 6.58 gprot/L, diluted 100-fold) and 293T cell (protein concentration 1.2 gprot/L, diluted 10-fold) according to the protocol, the results are as follows:



15. 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 the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside 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. 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 estimate 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.