（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

**Catalog No：E-BC-F043**

**Specification：48T(32 samples)/96T(80 samples)**

**Measuring instrument：Fluorescence Microplate Reader**

**(Ex/Em=535 nm/587 nm)**

**Detection range：0.03-1.5 U/L**

# **Elabscience®**Aspartate Aminotransferase (AST/GOT) Activity Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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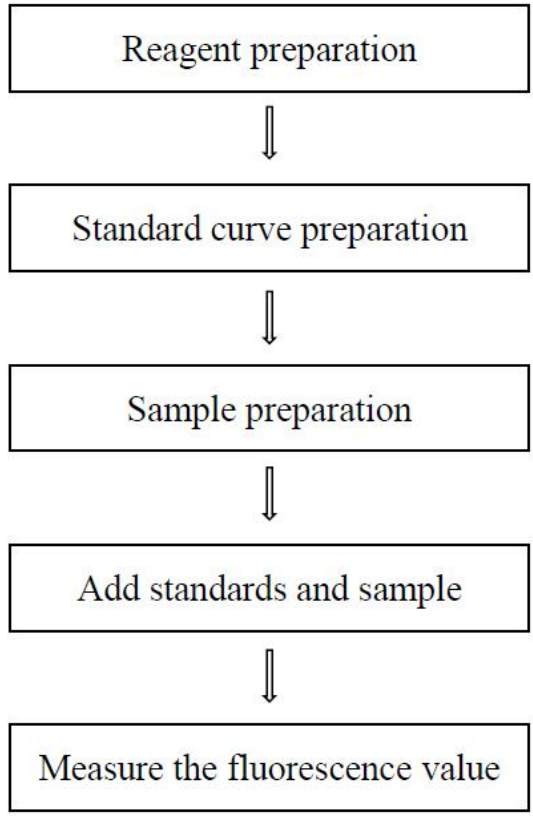
**Assay summary**



## Intended use

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

## 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 in the cytoplasm into the blood and the increase of serum AST activity. AST can catalyze the substrate to produce pyruvate. Pyruvate is oxidized to produce hydrogen peroxide, which causes the fluorescent probe to produce fluoresce. The activity of AST can be calculated by measuring the increase of fluorescence value at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

## 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℃, 12 months |
| Reagent 2 | Substrate | 1.5 mL × 1 vial | 1.5 mL × 2 vials | -20℃, 12 months, shading light |
| Reagent 3 | Enzyme Reagent | Powder × 1 vial | Powder × 2 vials | -20℃, 12 months, shading light |
| Reagent 4 | Aqueous Alkali | 0.3 mL × 1 vial | 0.6 mL × 1 vial | -20℃, 12 months, shading light |
| Reagent 5 | 100 mmol/L Pyruvate Standard | 1 mL × 1 vial | 1 mL × 1 vial | -20℃, 12 months, shading light |
| | Black Microplate | 96 wells | | No requirement |
| | Plate Sealer | 2 pieces | | |

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

**Instruments:**

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

## Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 1.2 mL of buffer solution, mix well to dissolve. 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 is advised.

③ The 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 on spot and the prepared solution should be used up within 1 h.

④ The 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 on spot.

⑤ The 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 on spot.

⑥ The preparation of standard curve：
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 a serial concentration. The recommended dilution gradient is as follows: 80, 60, 50, 40, 30, 20, 10, 0 μmol/L. Reference is as follows:

| 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 |

## Sample preparation

### ① Sample preparation

**Serum and plasma:** Detect the sample directly. If the sample contains a lot of lipids, chylous, etc., it can be centrifuged at 5000×g at 4°C for 5 min, take the clarified part and store it on ice for testing.

**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℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

**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 a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

### ② Dilution of sample

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

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

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

## The key points of the assay

Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

## Operating steps

① Standard well: add 20 μL of standard with different concentrations into the corresponding well..

Sample well: add 20 μL of sample into the corresponding well.

② Add 100 μL of reaction working solution to each well.

③ Mix fully with microplate reader for 5 s and stand at room temperature for 3 min.

④ Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, recorded as $F_1$, and then react at room temperature for 60 min with shading light. The fluorescence intensity of each well was determined under the same wavelength, and recorded as $F_2$, then $\Delta F = F_2 - F_1$ ( Note: There is no change in fluorescence value of standard well, plot the standard curve with the fluorescence value of $F_2$(standard)).

## 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 absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve ($\mathbf{y = a_1x + b_1}$) with graph software (or EXCEL).

**The sample**

**1. Serum (plasma) sample:**

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

$$\text{AST activity (U/L)} = \frac{\Delta F\text{-}b}{a} \div T \times f$$

**2. Tissue and cell samples:**

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

$$\text{AST activity (U/gprot)} = \frac{\Delta F\text{-}b}{a} \div T \times f \div C_{Pr}$$

**[Note]**

ΔF: The absolute fluorescence value of sample, $\triangle F = F_2 - F_1$.

T: The reaction time, 60 min.

f: Dilution factor of sample before tested.

Cpr: Concentration of protein in sample, gprot/L.

# Appendix I Performance Characteristics

## 1. 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

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 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 |

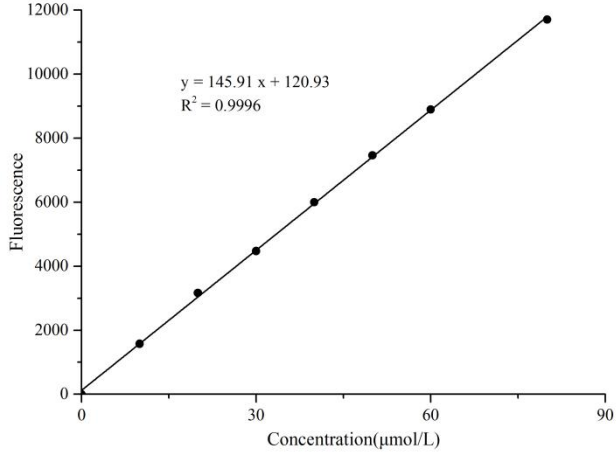
### Sensitivity

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

**2. 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), so the standard curve and data are provided as below for reference only:

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



# Appendix II Example Analysis

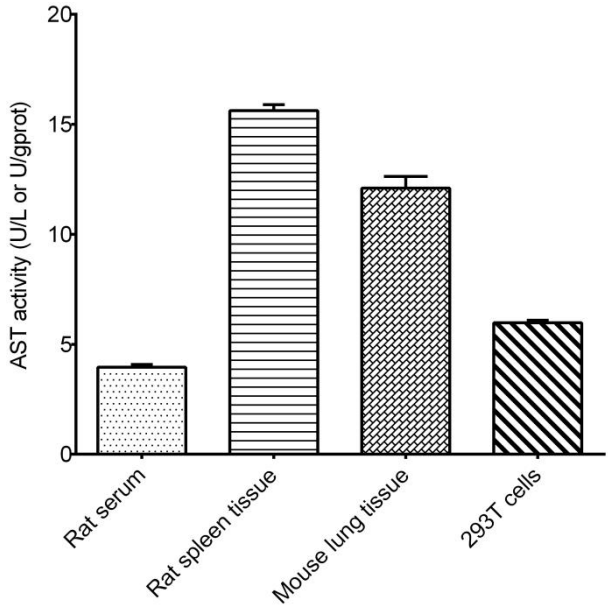
**Example analysis:**

For 10% rat spleen tissue, dilute for 80 times, take 20 μL for detection, and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 96.281 x + 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

Detect rat serum (dilute for 10 times), 10% rat spleen tissue homogenate (the concentration of protein is 6.90 gprot/L, dilute for 80 times), 10% mouse lung tissue homogenate (the concentration of protein is 6.58 gprot/L, dilute for 100 times) and 293T cell (the concentration of protein is 1.2 gprot/L, dilute for 10 times) according to the protocol, the result is as follows:



# Appendix III Publications


1. Fang X, Ding H, Chen Y, et al. Wireless Optogenetic Targeting Nociceptors Helps Host Cells Win the Competitive Colonization in Implant-Associated Infections[J]. Small Methods, 2024, 8(12): 2400216.
2. Ma X, Zhang W, Chen Y, et al. Paeoniflorin inhibited GSDMD to alleviate ANIT-induced cholestasis via pyroptosis signaling pathway[J]. Phytomedicine, 2024, 134: 156021.
3. Cao C, Liu W, Guo X, et al. Identification and validation of efferocytosis-related biomarkers for the diagnosis of metabolic dysfunction-associated steatohepatitis based on bioinformatics analysis and machine learning[J]. Frontiers in Immunology, 2024, 15: 1460431.
4. Popa A, Usatiuc L O, Scurtu I C, et al. Assessing the Anti-Inflammatory and Antioxidant Activity of Mangiferin in Murine Model for Myocarditis: Perspectives and Challenges[J]. International Journal of Molecular Sciences, 2024, 25(18): 9970.Y
5. Kaboutari M, Asle-Rousta M, Mahmazi S. Protective effect of menthol against thioacetamide-induced hepatic encephalopathy by suppressing oxidative stress and inflammation, augmenting expression of BDNF and α7-nACh receptor, and improving spatial memory[J]. European Journal of Pharmacology, 2024, 981: 176916.
6. Zhao Y, Wang R, Li A, et al. Protective effect of hydroxysafflor yellow a on thioacetamide-induced liver injury and osteopenia in zebrafish[J]. Toxicology and Applied Pharmacology, 2024, 492: 117109.

## Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.