



# **Aspartate Transaminase Microplate Assay Kit User Manual**

**Catalog # FTA0004**

(Version 1.2F)

Detection and Quantification of Aspartate Transaminase (AST)  
Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell  
culture media and Other biological fluids Samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

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## I. INTRODUCTION

Aspartate Transaminase (AST), also known as serum glutamic oxaloacetic transaminase (GOT) or aspartate aminotransferase (ASAT/AAT), facilitates the conversion of aspartate and  $\alpha$ -ketoglutarate to oxaloacetate and glutamate. There are two isoenzymes in humans: GOT1 is a cytosolic isoenzyme derived from red blood cells and heart; GOT2 is the mitochondrial isoenzyme found mainly in the liver. AST is elevated in liver and muscle diseases. It is part of diagnostic tests for liver function, myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease and trauma.

The enzyme catalysed reaction product phenylhydrazone can be measured at a colorimetric readout at 520 nm.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	10 ml x 1	4 °C
Dye Reagent I	10 ml x 1	4 °C
Dye Reagent II	20 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

### Note:

**Substrate:** add 10 ml Substrate Diluent to dissolve before use.

**Standard:** add 1 ml Assay Buffer to dissolve before use, it will be 20 µmol/ml.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 520 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

#### IV. SAMPLE PREPARATION

##### 1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

##### 2. For tissue samples

Weigh out 0.1g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

##### 3. For serum or plasma samples

Detect directly.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	10 $\mu$ l	--	--	--
Assay Buffer	--	--	10 $\mu$ l	10 $\mu$ l
Standard	--	--	10 $\mu$ l	--
Substrate	50 $\mu$ l	50 $\mu$ l	40 $\mu$ l	50 $\mu$ l
Mix, put it in the oven, 37 °C for 30 minutes.				
Dye Reagent I	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l
Dye Reagent II	90 $\mu$ l	90 $\mu$ l	90 $\mu$ l	90 $\mu$ l
Sample	--	10 $\mu$ l	--	--
Mix, record absorbance measured at 520 nm.				

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

## VI. CALCULATION

**Unit Definition:** One Unit of AST activity is defined as the enzyme produces 1  $\mu\text{mol}$  of pyruvic acid per hour.

### 1. According to the volume of serum or plasma

$$\begin{aligned}\text{AST (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &/T \\ &= 40 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

### 2. According to the weight of sample

$$\begin{aligned}\text{AST (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 40 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

### 3. According to the quantity of cell or bacteria

$$\begin{aligned}\text{AST (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 40 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

$C_{\text{Standard}}$ : the concentration of standard, 20  $\mu\text{mol/ml}$ ;

$W$ : the weight of sample, g;

$N$ : the quantity of cell or bacteria,  $N \times 10^4$ ;

$V_{\text{Standard}}$ : the volume of standard, 0.01 ml;

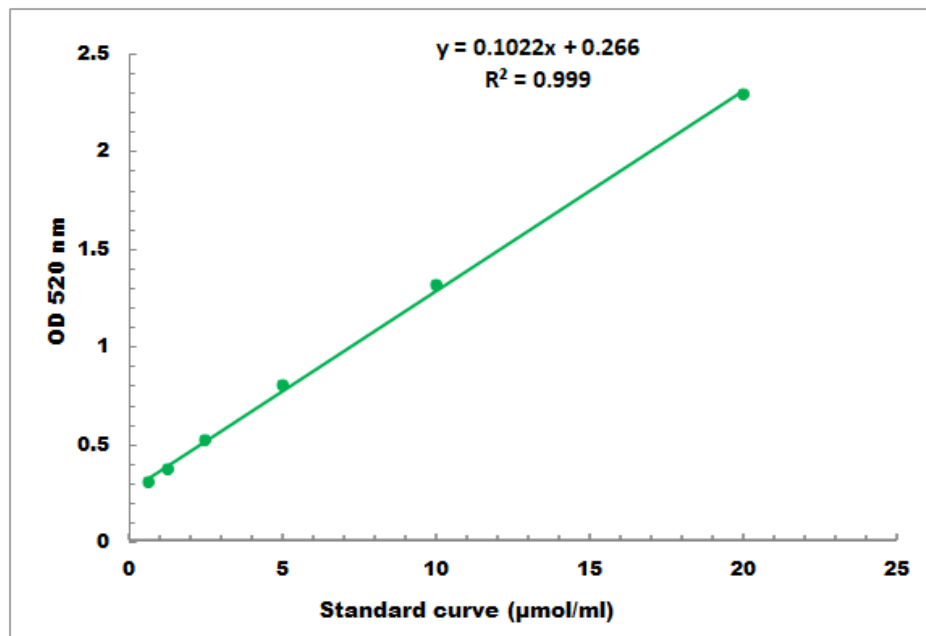
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

$T$ : the reaction time, 0.5 hour.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.625 μmol/ml - 20 μmol/ml

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES