

Aspartate Transaminase Microplate Assay Kit

Basic information:

Catalog No.: UAK1004 Size: 100 Assays

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

I. INTRODUCTION

Aspartate Transaminase (AST), also known as serum glutamic oxaloacetic transaminase (GOT) or aspartate aminotransferase (ASAT/AAT), facilitates the conversion of aspartate and α -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	

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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 $\mu\text{mol/ml}$.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 520 nm
2. Distilled water
3. 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 5×10^6 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

Reagent	Sample	Control	Standard
Sample	10 µl	--	--
Assay Buffer	--	--	10 µl
Standard	--	--	10 µl
Substrate	50 µl	50 µl	40 µl
Mix, put it in the oven, 37 °C for 30 minutes.			
Dye Reagent I	50 µl	50 µl	50 µl
Dye Reagent II	90 µl	90 µl	90 µl
Sample	--	10 µl	--

VI. CALCULATION

Unit Definition: One Unit of AST activity is defined as the enzyme produces 1 µmol 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

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

3. According to the quantity of cell or bacteria

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

C_{Standard} : the concentration of Standard, 20 µmol/ml;

W: the weight of sample, g;

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

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

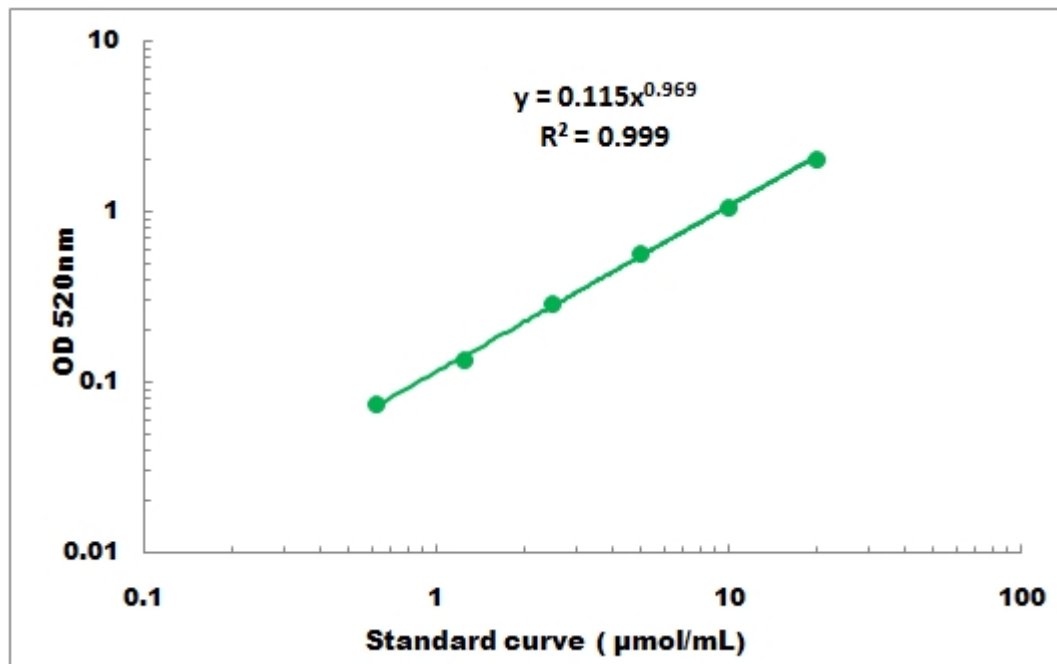
V_{Sample} : the volume of sample, 0.01 ml;

V_{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