Aspartate Transaminase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6613

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 .

1. Introduction

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

Aspartate Transaminase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining Aspartate Transaminase activity in various samples. The en zyme catalysed reaction product phenylhydrazone can be measured at a colorimetric readout at 520 nm.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30 mL x 4	4 °C
Substrate	Powder x 1	4 °C, keep in dark
Dye Reagent I	5 mL x 1	4 °C
Dye Reagent II	10 mL x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Add 1 mL Assay Buffer to dissolve before use , it will be 2 0 μmol/ml as the top standard solution. Store at 4°C for 1 month after reconstitution. Perform 2 -fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 20/10/5/2.5/1.25/ 0.625 μmol/ml.

Substrate: Add 5 mL Assay Buffer to dissolve before use. Keep in dark and store at 4°C for 1 week.

Positive Control: Briefly centrifuge prior to opening. Add 1 mL Assay Buffer to dissolve before use. Store at -20°C.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. 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% , sonicate 3s, interval 10s , repeat 30 times); centrifuged at 8000 g 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 8000 g 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.

6. Assay Procedure

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	10 μl	--	--	--	--
Assay Buffer	--	10 μl	--	--	--
Standard	--	--	10 μl	--	--
Distilled Water	--	--	--	10 μl	--
Positive Control	--	--	--	--	10 μl
Substrate	50 μl	50 μl	50 μl	50 μl	50 μl
Mix, cover the plate adhesive strips, put it into the oven, 37 °C for 30 minutes.					
Dye Reagent I	50 μl	50 μl	50 μl	50 μl	50 μl
Dye Reagent II	90 μl	90 μl	90 μl	90 μl	90 μl
Mix, record absorbance measured at 520 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

7. Calculation

Unit Definition:

One Unit of AST activity is defined as the enzyme produces 1 μmol of pyruvic acid per minute .

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times n \ (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times C_{Protein} \times T} \ (U/mg)$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \ (U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \ (U/g)$$

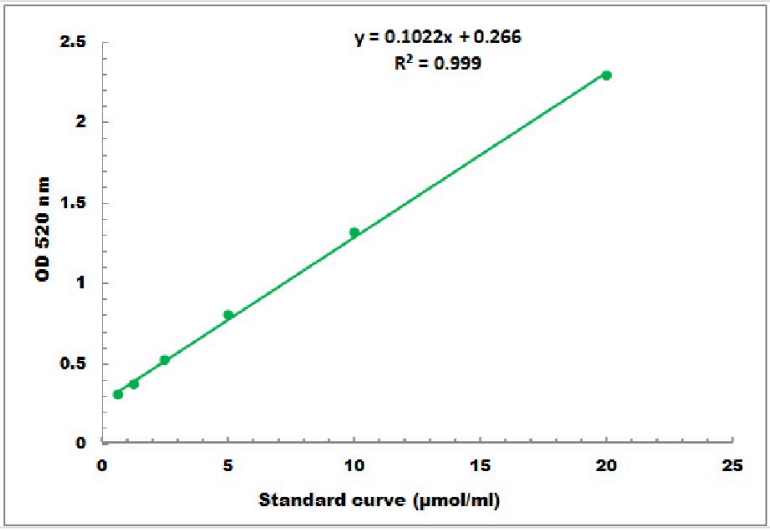
2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \ (U/mL)$$

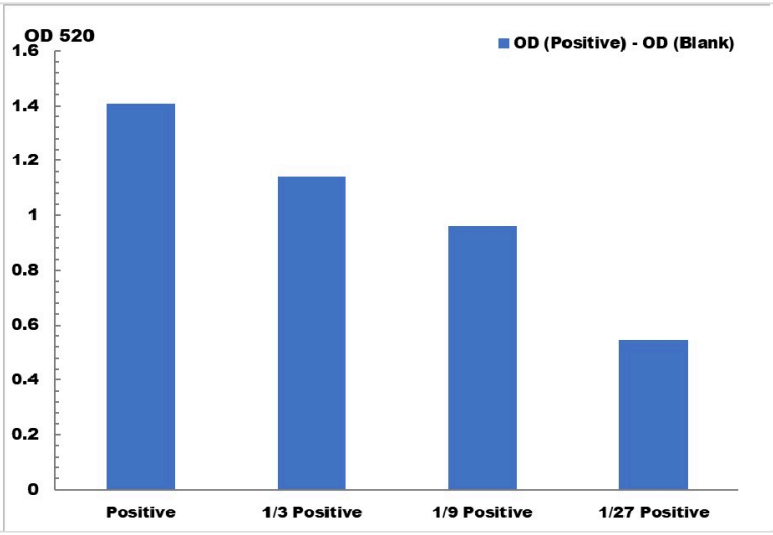
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, μmol/mL
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer in sample preparation , mL
T	the reaction time , minute

8. Typical Data

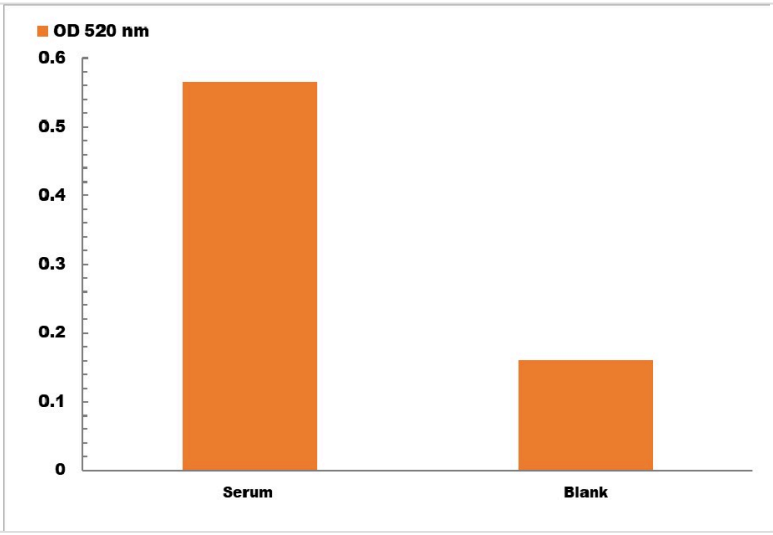
The standard curve is for demonstration only. A standard curve must be run with each assay.



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



Positive Control reaction in 96 -well plate assay with decreasing the concentration



Determination of aspartate transaminase in serum