# Aspartate Transaminase

# Microplate Assay Kit

# User Manual

**Catalog # CAK1004**

(Version 1.5H)

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.**

## I. 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.

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 | 5 ml x 1 | 4 °C |
| 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 | |

**Note:**

**Substrate:** add 5 ml Substrate Diluent to dissolve before use.

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

**Positive Control**: add 1 ml Assay Buffer to dissolve before use.

## 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%, sonicate 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 | 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:**

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 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.

3) Reagents must be added step by step, can not be mixed and added together.

## VI. 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 volume of serum or plasma

$$AST\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.667 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

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

$$= 0.667 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 0.667 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: 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;

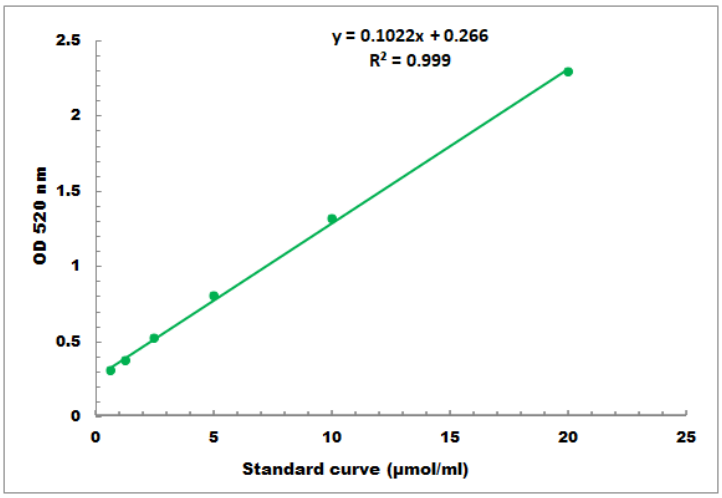
$V_{Sample}$: the volume of sample, 0.01 ml;

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

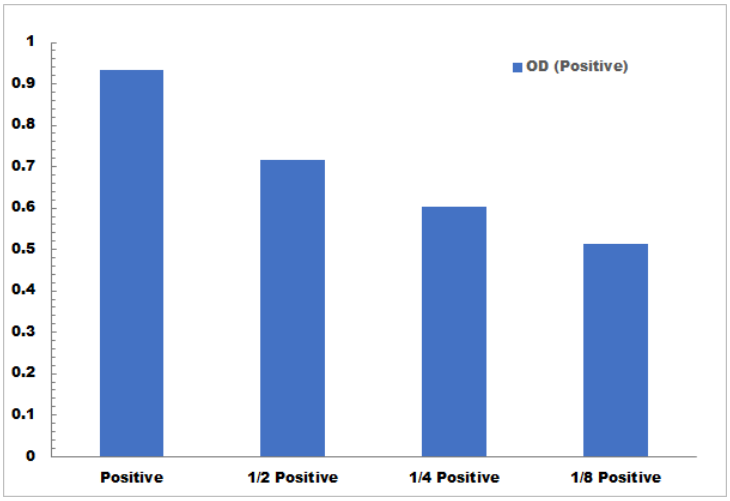
T: the reaction time, 0.5 hour = 30 minutes.

## 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




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

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

## IX. NOTES