Aspartate Aminotransferase Activity Colorimetric Assay
(Catalog: TBS2013, 100 Assays, Store at -20 °C)

DESCRIPTION

Aspartate Aminotransferase (EC2.6.1.1, AST), also known as glutamic-oxaloacetic transaminase (GOT), is an enzyme that catalyzes the reversible transfer of an amino group from aspartate. This reaction is a key part of the nitrogen metabolism and the citric acid cycle, connecting amino acid metabolism to energy production. AST is found in various tissues, particularly in liver (high concentration), heart (cardiac muscle), skeletal muscle, kidneys and brain. Cytoplasmic AST and mitochondrial AST are found in circulation. When elevated or reduced in the blood, it can indicate metabolic disorders. So it is a key marker in diagnosing or monitoring these conditions.

Tribio™ Aspartate Aminotransferase Activity Colorimetric Assay is based on a dehydrogenase coupled reaction converting chromophore to a colored formazan, which can be measured at OD 460 nm. The generated signal is proportional to the AST enzyme activity. The kit provides the easiest and most accurate approach to measuring AST activity from a variety of samples.

Synonyms: AST; GOT; SGOT; ASAT; AAT.

APPLICATIONS

Specifically detect aspartate aminotransferase from cells and biological tissues.

KIT CONTENTS FOR 100 TESTS:

Name	Part Size
Assay Buffer	10 mL
Enzyme mix	0.7 mL
Enzyme substrate I (9x)	1.2 mL
Enzyme substrate II (20x)	0.6
Enzyme substrate III (10x)	1.1 mL
Detection Probe (10x)	1.1 mL
Standard stock (10 mM)	0.3 mL
Positive control (5x)	70 µL
Co-factor (25x)	0.5 mL

Storage conditions: Store the Reagent at –20 °C for a year.

PROCEDURES

1. Sample preparation

1) Wash the cells with 400 µL cold PBS.
2) Pellet 2 X 10^5 cells at 2000 rpm for 5 min.
3) Aspirate the wash solution from the tube.
4) Homogenization in 150 µL assay buffer for 30 sec.

(For tissues, weigh ~20 mg tissue & wash with cold PBS. Homogenize in Assay Buffer in a micro-centrifuge tube).

5) Centrifuge at 14000 rpm for 5 min.
6) Transfer the extracted supernatant into 2 labeled tubs.
7) To transfer 30 µL of extracted samples into labeled 96-well plate.

2. Prepare standards as below Table 1.

Table 1: Glutamate Standard Preparation

Std	Standard (µL)	Assay buffer (µL)	µM	nmol/well
1	100 (10 mM stock)	400	2000	60
2	150 (Std1)	150	1000	30
3	150 (Std1)	150	500	15
4	150 (Std1)	150	250	7.5
5	150 (Std1)	150	125	3.75
6	150 (Std1)	150	62.5	1.875
7	150 (Std1)	150	31.25	0.9375
8	0	150	0	0

3. Prepare premix for 40 µl/well: 11 µL of substrate I (9x) + 5 µL of substrate II (20x) + 10 µL of substrate III (10x) + 10 µL of detection probe (10x) + 4 µL of co-factor (25x).

4. Set up reaction
Add 30 µL of Standard or test sample preparation, and positive control or blank (assay buffer) into the indicated well. (Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range).

5. Add 30 µL of enzyme mix to each well containing the standards, test samples, positive control and blank.

6. Add 40 µL of premix to each well containing the standards, test samples, positive control and blank.

7. Initiate Reaction:
Incubate at 37°C for about 30-60 minutes, with gentle shaking and protecting from light.

8. Measure OD value at 460 nm:
The glutamate standard and samples can be read at the end point.

9. Calculation using standard curve:
The standard values subtract the blank value (0 µM Standard) and plot the ΔOD against standard concentrations. Determine the slope and calculate the AST concentration of the sample using the equation obtained from the linear regression of the standard curve.

AST Activity (U/L) = DF * (ODBLANK – OD SAMPLE)/ (t * Slope)

Where: R sample and R blank are optical density readings of the sample and blank, respectively. DF is the sample dilution factor. Slope is the linear regression fit of the standard points and t is the reaction time (30 or 60 min).

Note1: If unknown sample results are over standard curve range, dilute samples with assay buffer, and repeat the assay.

Unit definition: 1 Unit (U) definition that AST converse of 1 µmole of substrate to glutamate per min at 37°C.

TYPICAL STANDARD CURVE

The Typical Standard curve showed as an example in Fig.1. It can be used only for reference.

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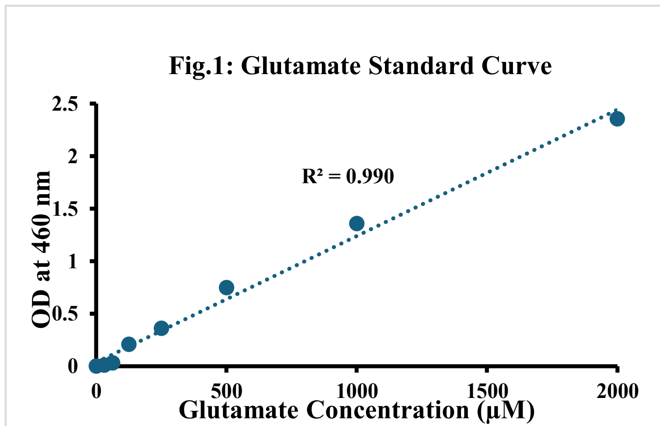
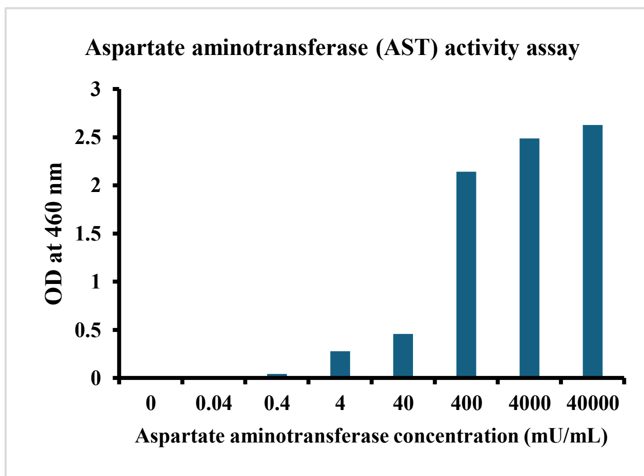


The positive control graph is shown in Fig.2 as below.



RELATIVE PRODUCTS
Resazurin Cell Viability Kit (TBS2001)
LDH Cytotoxicity Assay (TBS2002)
Caspase-3 Colorimetric Assay kit (TBS2030)
CD38 Cyclase Activity Assay (TBS2100)
LDH Activity Assay (TBS2012)
CCK-8 Cell Viability Assay (TBS2022)
GOT Activity Assay (TBS2013)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Homocysteine Fluorometric Assay (TBS2091)
AHCY Inhibitor Screening Assay (TBS2097)
G6PDH Activity Colorimetric Assay (TBS2102)
ATP Colorimetric / Fluorometric Assay (TBS2010)
ADP Colorimetric / Fluorometric Assay (TBS2020)
NNMT Activity Assay (TBS2098)
NADP Assay (TBS2058)
NAD/NADH Assay (TBS2029)

For research use only.