Technical Bulletin

Aspartate Transaminase (AST) Assay Kit

Catalogue Number MAK467

Product Description

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 testing for liver function, myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, and trauma.

Simple, direct and automation-ready procedures for measuring AST activity find wide applications in research and drug discovery. The Aspartate Transaminase (AST) Assay Kit is based on the quantification of oxaloacetate produced by AST. In this assay, oxaloacetate and NADH are converted to malate and NAD+ by the enzyme malate dehydrogenase. The decrease in NADH absorbance at 340 nm is proportionate to AST activity.

The linear detection range of the kit is 2-100 units per liter (U/L). The kit is suitable for AST activity determination in serum, plasma, and other biological samples, as well as for studying the effects of drugs on AST activity.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 24 mL
  Catalogue Number MAK467A
- Cofactor 120 µL
  Catalogue Number MAK467B
- Enzyme Mix 120 µL
  Catalogue Number MAK467C
- NADH Reagent 1 vial
  Catalogue Number MAK467D

Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store all components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to assay temperature (room temperature or 37 °C) prior to use.

NADH Reagent: Reconstitute vial with 1000 µL of purified water to generate a 10 mM standard solution. Reconstituted NADH reagent is stable for three weeks when stored frozen at -20 °C.

Assay Buffer: Ready to use. Mix well by vigorous shaking prior to use

Enzyme mix: Ready to use. Keep thawed enzyme on ice.

Cofactor: Ready to use. Keep thawed enzyme on ice.

Procedure

All samples and standards should be run in duplicate.

Assays can be performed at 37 °C or at room temperature. Prior to assay, bring the working reagents, microplate and spectrophotometer to the desired temperature.

Note: The NADH Reagent acts as the standard when preparing the standard wells.

Procedure Using 96-well plate

Sample Preparation

1. The assay is compatible with serum or plasma treated with EDTA or heparin. Samples should be clear and free of particles or precipitates. Hemolyzed samples should not be used.
2. Transfer 20 µL of each sample into separate wells of a clear, flat-bottom 96-well plate.
3. For each assay plate, also include two wells with 20 µL of purified water each to be used for the NADH Standard and Blank.
4. Keep plate at the desired assay temperature (such as, 37 °C).

Working Reagents

1. Mix enough reagents for the number of assays to be performed. Prepare Working Reagents according to Table 1.
    a. For each NADH Standard and Sample well, prepare 206 µL of Working Reagent.
    b. For each Blank well, prepare 206 µL of Blank Working Reagent.

Table 1.
Preparation of Working Reagents for 96-Well Plate Assay

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	200 µL	200 µL
Cofactor	1 µL	1 µL
Enzyme Mix	1 µL	1 µL
NADH Reagent	4 µL	-
Purified Water	-	4 µL

2. Warm Working Reagents to desired assay temperature.
3. Add 200 µL of Working Reagent to each Sample and NADH Standard well. Add 200 µL of Blank Working Reagent to the Blank well. Tap plate to mix.

Measurement

Immediately tap plate to mix. Incubate at the desired temperature and read optical density (OD) at 340 nm at 5 minutes and at 10 minutes. Alternatively, record optical density (OD) in kinetic mode at 340 nm.

Procedure Using Cuvettes

1. For each assay, include one Standard and one Blank control.
2. For each NADH Standard and Sample well, prepare 1030 µL of Working Reagent according to Table 2.

Table 2.
Preparation of Working Reagents for Cuvette Assay

Reagent	Working Reagent
Assay Buffer	1000 µL
Cofactor	5 µL
Enzyme Mix	5 µL
NADH Reagent	20 µL

3. Transfer 990 µL of Working Reagent to each Sample and Standard cuvette.
4. To each Blank cuvette, add 960 µL Assay Buffer, 5 µL Cofactor, 5 µL Enzyme mix, and 20 µL purified water (total volume 990 µL).
5. Warm to desired assay temperature (such as, 37 °C).
6. Add 100 µL of Sample to the Sample Cuvette.
7. Transfer 100 µL of purified water to the Standard and Blank Control cuvettes, respectively. Mix immediately.
8. Incubate at the desired temperature and read optical density (OD) at 340 nm at 5 minutes and at 10 minutes. Alternatively, record optical density (OD) in kinetic mode at 340 nm.

Results

1. For each Sample, calculate the rate of NADH consumption by subtracting the OD at 10 minutes from the OD at 5 minutes (ΔOD_S).
2. Calculate the rate for the NADH Standard by subtracting the OD at 10 minutes from the OD at 5 minutes (ΔOD_{STD}).

3. For the 96-well Plate Assay, determine ALT activity using the following equation:

AST (U/L) =

$$388 \text{ x } \left(\frac{\Delta OD_S - \Delta OD_{STD}}{OD_{STD} - OD_{BLANK}}\right)$$

where:

OD_{STD} = 5 minute OD reading at 340 nm of the NADH Standard

OD_{BLANK} = 5 minute OD reading at 340 nm of the Blank

388 (Factor) =

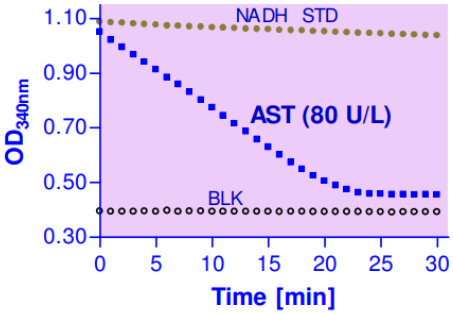
$$10mM\ NADH\ x\ \frac{4\ \mu L\ Vol._{NADH}}{206\ \mu L\ Vol._{WR}}\ x\ \frac{200\ \mu L\ Vol._{WR}}{220\ \mu L\ Vol._{Total}}\ x \frac{11\ (Sample\ dilution)}{5\ minutes}$$

= 388 µM/minute

If the calculated AST activity is higher than 100 U/L, dilute the Sample in Assay Buffer and repeat assay. Multiply results by the dilution factor.

Unit definition: 1 Unit (U) of AST will catalyze the conversion of 1 µmole of aspartate to oxaloacetate per minute at pH 8.1.

Example of reaction with purified AST enzyme (assayed at 37 °C)



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mak467pis Rev 09/25