Aspartate Transaminase (AST) Assay Kit (Colorimetric)

LS-K297-100 (100 Tests) • **Store at -20°C**

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.

Simple, direct and automation-ready procedures for measuring AST activity find wide applications in research and drug discovery. LSBio's' AST activity assay 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.

Key Features

- Sensitive. Linear detection range: 2–100 U/L.
- Simple and convenient. This simple, convenient assay can be carried out in a microplate or a cuvette and takes only 10 min.

Applications

- Direct Assays: AST activity in serum, plasma and other biological samples.
- Drug Discovery/Pharmacology: effects of drugs on AST activity.

Components

Component	K297-100
	100 Tests
Assay Buffer	24 mL
Cofactor	120 µL
Enzyme Mix	120 µL
NADH Reagent	Dried

Materials Not Supplied

Pipetting devices and accessories. Clear bottom 96-well plates (e.g. Corning Costar) and plate reader or spectrophotometer and cuvettes for measuring OD340nm.

Storage

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

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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Assay Procedure

Equilibrate all components to room temperature. Reconstitute the NADH Reagent tube with 1000 µL dH_2O (final 10 mM). Unused reconstituted NADH reagent is stable for three weeks when stored frozen at -20°C. Mix assay buffer well by vigorous shaking. Keep thawed enzyme on ice.

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.

Assay is compatible with serum or plasma (heparin, EDTA). Samples should be clear and free of particles or precipitates. Hemolyzed samples should not be used.

Procedure using 96-well plate

1. Samples and controls. Transfer 20 µL sample to each well. For each assay plate, include two wells with 20 µL dH2O to be used for the NADH Standard and Blank. Keep plate at the desired temperature (e.g. 37°C).
2. Prepare Working Reagent for Sample and Standard wells, by mixing for each well, 200 µL Assay Buffer, 1 µL Cofactor, 1 µL Enzyme Mix and 4 µL NADH. Warm to desired temperature (e.g. 37°C).

   Prepare Blank Reagent for the Blank well, by mixing 200 µL Assay Buffer, 1 µL Cofactor, 1 µL Enzyme Mix and 4 µL dH2O. Warm to desired temperature (e.g. 37°C).
3. Add 200 µL Working Reagent to the Standard and Sample wells, and 200 µL Blank Reagent to the Blank well.

   Immediately tap plate to mix, incubate at the desired temperature and read OD340nm at 5 min and at 10 min. Alternatively, record kinetics at 340 nm.

Procedure using cuvettes

1. For each assay, include one Standard and one Blank control.

   For each Sample and Standard, prepare Working Reagent by mixing 1000 µL Assay Buffer, 5 µL Cofactor, 5 µL Enzyme Mix and 20 µL NADH. Transfer 990 µL Working Reagent to each sample cuvette and standard cuvette. Warm to desired temperature (e.g. 37°C).

   To Blank control cuvette, add 960 µL Assay Buffer, 5 µL Cofactor, 5 µL Enzyme Mix and 20 µL dH2O. Warm to desired temperature (e.g. 37°C).
2. Prewarm sample to the desired temperature. Add 100 µL Sample to the Sample Cuvette. Transfer 100 µL H2O to the Standard cuvette and to Blank Control cuvette, respectively.
3. Mix immediately. Read OD340nm at 5 min and 10 min., alternatively, record kinetics at 340 nm.

Calculations

For each Sample, calculate the rate of NADH consumption by subtracting the OD at 10 min from the OD at 5 min (ΔOD_S). Similarly, calculate the rate (ΔOD_{NADH}) for the NADH standard ($OD_{5min} - OD_{10min}$).

Determine AST activity using the following equation,

$$AST = 388 \times \frac{\Delta OD_S - \Delta OD_{NADH}}{OD_{STD} - OD_{BLK}} \quad (U/L)$$

OD_{STD} and OD_{BLK} are the OD_{340nm} values of NADH Standard and Blank at 5 min, respectively.

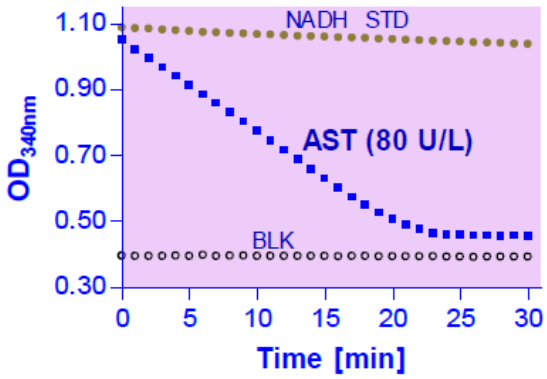
The factor 388 is derived from

$$\text{Factor} = 10\ \text{mM NADH} \times \frac{4\ \mu L\ Vol._{NADH}}{206\ \mu L\ Vol._{WR}} \times \frac{200\ \mu L\ Vol._{WR}}{220\ \mu L\ Vol._{Total}} \times \frac{11\ (\text{sample dilution})}{5\ \text{min}}$$

$$= 388\ \mu M/\text{min}$$

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

Sample Data



Purified AST enzyme (assayed at 37°C)

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