Aspartate Transaminase Assay (AST)

Cat. No. 8608
100 Tests in 96-well plate

Introduction

Aspartate Aminotransferase (AST), also known as serum glutamate-oxaloacetate transaminase (SGOT), catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate. The products of this transamination reaction are oxaloacetate and glutamate. AST is found in liver, heart, skeletal muscle, kidneys, brains and red blood cells. Significantly elevated serum AST levels often suggest the existence of medical problems, such as myocardial infarction, acute pancreatitis, acute renal disease, and trauma. This colorimetric assay is based on the oxidization of NADH to NAD in the presence of oxaloacetate and malate dehydrogenase. AST activity is determined by assaying the rate of NADH oxidation, which is proportional to the reduction in absorbance at 340nm over time (ΔOD_{340nm}/min).

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8608a	1	Assay buffer	25 mL	4 °C
8608b	1	AST positive control	20 µL	-20°C
8608c	1	NADH	0.2 mL	-20°C
8608d	1	Cofactor	0.2 mL	-20°C
8608e	1	Enzyme	0.1 mL	-20°C
8608f	1	Substrate	1.0 mL	-20°C

Product Use

The Aspartate Aminotransferase Assay kit measures the aspartate aminotransferase activity in different types of samples, such as serum, plasma, and tissues. This product is for research purposes only and is not approved for use in animals, humans, or diagnostic procedures.

Quality Control

Diluted AST positive control is measured with the AST Assay kit at different reaction times (Figure 1 and 2). The detection range is from 0.02 to 0.2 U/mL.

Shipping

Shipped on dry ice.

Reagents and Positive Control Preparation

1. Diluted AST positive control: Add 1 µl of AST positive control into 99 µl of assay buffer (8608a). Prepare diluted AST positive control to a final volume of 10 µL/well on a 96-well flat bottom plate.

Procedures (96-well plate)

A. Preparation of test samples and blank

1. Cells or tissues can be homogenized in 4 volumes of the assay buffer (8608a). Centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serial diluted to ensure that the readings are within the range of the standard curve. Prepare test samples to a final volume of 10 µL/well on a 96-well flat bottom plate.
3. Prepare blank by adding 10 µL of assay buffer (8608a) into one well of the 96-well flat bottom plate.

B. Working reagent preparation and measurements

1. Prepare the appropriate volume of AST assay working reagent based on the number of samples to be measured. For each well to be assayed, prepare working reagent by mixing 100 µL assay buffer (8608a), 2 µL NADH (8608c), 2 µL cofactor (8608d), and 1 µL enzyme (8608e).
2. Add 100 µL of working reagent mix and 10 µL of substrate (8608f) into each well of the 96-well plate, which should now contain diluted AST positive control, samples, and blank. Immediately mix the reaction and start recording OD_{340nm} over 5 minute intervals, collecting data every 1 minute. Figure 1 shows data for AST positive control.

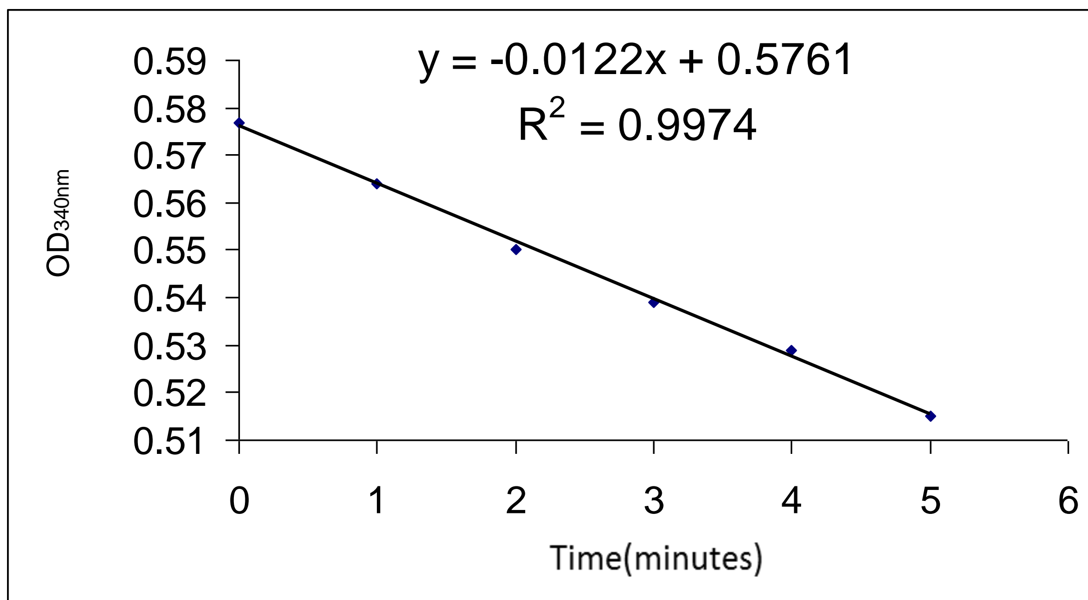

Figure 1. Change in absorbance of diluted AST positive control at 340nm.

C. Calculations

1. Determine the change in absorbance (ΔOD_{340nm}/min) by plotting the absorbance value at ΔOD_{340nm} as a function of reaction time, and then obtain the slope of the linear portion of the curve, as shown in Figure 2.

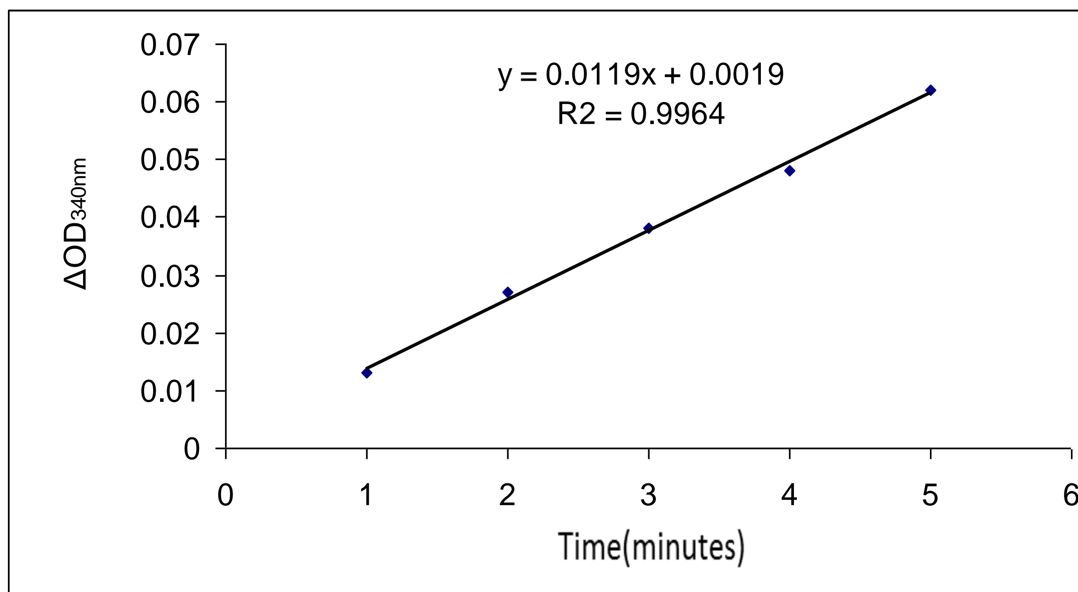

Figure 2. Change in absorbance ΔOD_{340nm} of diluted AST positive control during the time at 340nm.

2. Calculate AST activity using the following formula:

$$\text{AST (U/ml)} = \frac{\Delta OD_{340nm}/\text{min} \times 120\ \mu l}{1.94\ mM^{-1} \times 10\ \mu l} \times \text{sample dilution}$$

Note: The actual extinction coefficient for NADH at 340nm is $6.22 mM^{-1}cm^{-1}$. This value has been adjusted for the path length of the solution in a 96-well plate.

Unit definition: One unit converts 1.0 µmol of NADH to NAD^+ per minute at 25 ºC, at pH 7.4.

3. Use the formula below to calculate the activity of the AST positive control:

$$\text{AST (U/ml)} = \frac{0.0119 \times 120\ \mu l}{1.94\ mM^{-1} \times 10\ \mu l} \times 100 = 7.36\ \text{(U/ml)}$$