Alanine Transaminase Assay (ALT)

Cat. No. 8478
100 Tests in 96-well plate

Introduction

Alanine Aminotransferase (ALT), also known as serum glutamic-pyruvic transaminase (SGPT), catalyzes the reversible transfer of an amino group from alanine to α-ketoglutarate. The products of this transamination reaction are pyruvate and glutamate. ALT is found primarily in liver and serum, but occurs in other tissues as well. Significantly elevated serum ALT levels often suggest the existence of medical problems, such as hepatocellular injury, hepatitis, diabetes, bile duct problem and myopathy. This colorimetric assay is based on the oxidization of NADH to NAD in the presence of pyruvate and lactate dehydrogenase. The ALT 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
8478a	1	Assay buffer	10 mL	-20°C
8478b	1	ALT standard	10 µL	-20°C
8478c	1	Substrate mix	1.0 mL	-20°C
8478d	1	Cofactor	0.8 mL	-20°C
8478e	1	Enzyme	0.2 mL	-80°C

Product Use

The Alanine Transaminase Assay kit measures the alanine transaminase activity of different types of samples, such as serum, plasma and tissues. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Serially diluted alanine transaminase solutions with concentrations ranging from 0.03125 to 1.0 U/mL are measured with the ScienCell™ Alanine Transaminase Assay kit. The decrease in OD_{340nm} is monitored as a function of time (Figure 1) and the resulting standard curve of ΔOD_{340nm}/min vs alanine transaminase activity are plotted (Figure 2). A positive linear relationship between ΔOD_{340nm}/min & alanine transaminase activity can be observed.

Shipping and stability

Shipped on dry ice and stable for three months.

Procedure (96-well plate)

A. Preparation of uric acid standard

1. Add 1 µL of alanine transaminase standard (8478b) to 99 µL of assay buffer (8478a) to make a 100 µL solution of 2 U/mL alanine transaminase.
2. Obtain 7 test tubes, add 25 µL of assay buffer (8478a) into each tube and label them #1 through #7.
3. Add 25 µL 2 U/mL alanine transaminase into tube #1 and mix well to get the 1 U/mL alanine transaminase standard.
4. Transfer 25 µL of the 1 U/mL alanine transaminase standard from tube #1 to tube #2 and mix well to get the 0.5 U/mL alanine transaminase standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the alanine transaminase standards. Do not add any alanine transaminase to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each alanine transaminase standard by aliquoting 10 µL/well of each alanine transaminase standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	1 U/mL	0.5 U/mL	0.25 U/mL	0.125 U/mL	0.0625 U/mL	0.03125 U/mL	Blank
B	1 U/mL	0.5 U/mL	0.25 U/mL	0.125 U/mL	0.0625 U/mL	0.03125 U/mL	Blank

B. Preparation of test samples

1. Tissues can be homogenized in 4 volumes of the assay buffer (8478a). 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 make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 70 µL assay buffer (8478a), 10 µL substrate mix (8478c), 8 µL cofactor (8478d) and 2 µL enzyme mix (8478e).
2. Initiate the reaction by adding 90 µL of working reagent mix into each well of the 96-well plate containing alanine transaminase standard, samples and blank.
3. Follow the increases in OD_{340nm} using a plate reader and start recording OD_{340nm} over a 5 minutes interval, collecting data every 1 minute.

D. Calculations

1. Subtract the measured OD_{340nm} at different reaction time from the initial OD_{340nm} to obtain the corresponding ΔOD_{340nm} for each sample and alanine transaminase standard at different reaction time. Average the value of ΔOD_{340nm} of replicate wells. Subtract the measured ΔOD_{340nm} at different reaction time from the blank.
2. Based on the ΔOD_{340nm} of the alanine transaminase standard solutions, plotting the absorbance at ΔOD_{340nm}

as a function of reaction time (Figure 1) in which ΔOD_{340nm} /min is calculated.

3. Plot a standard curve of ΔOD_{340nm} /min vs alanine transaminase activity (Figure 2).
4. Calculate the alanine transaminase activity of test samples based on the standard curve.

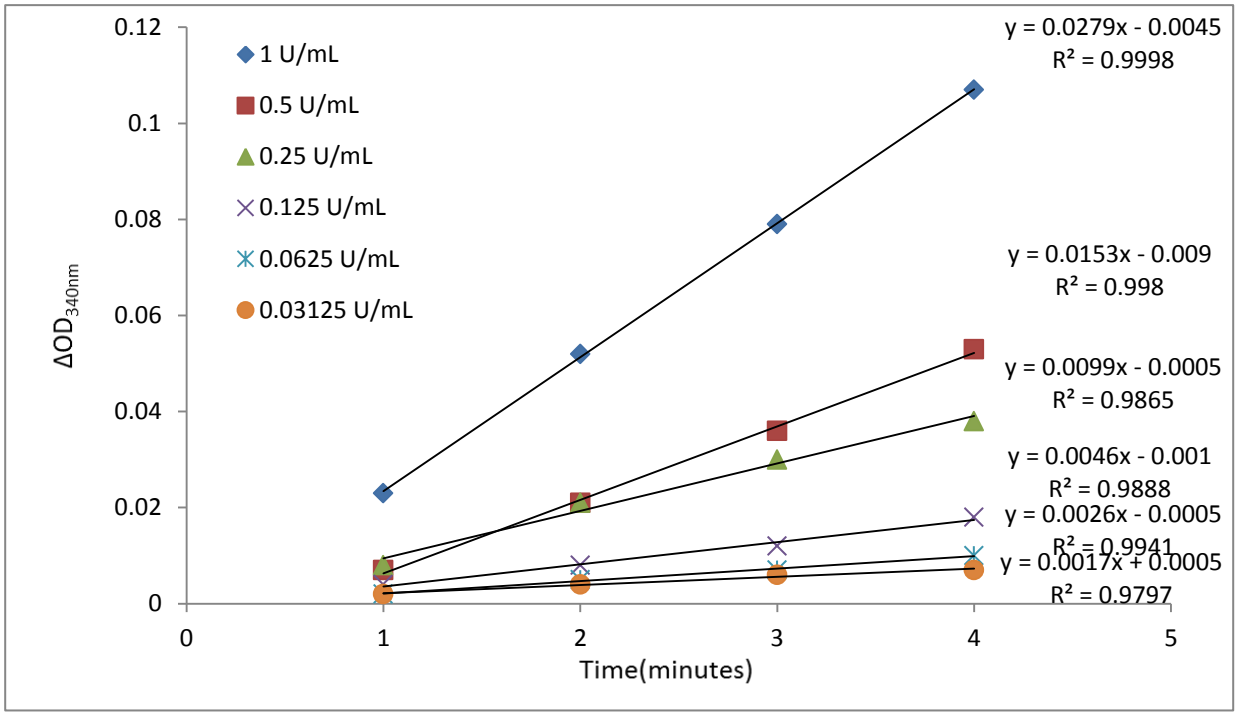

Figure1. Standard curves of ΔOD_{340nm} vs reaction time for alanine transaminase solution with different activity.

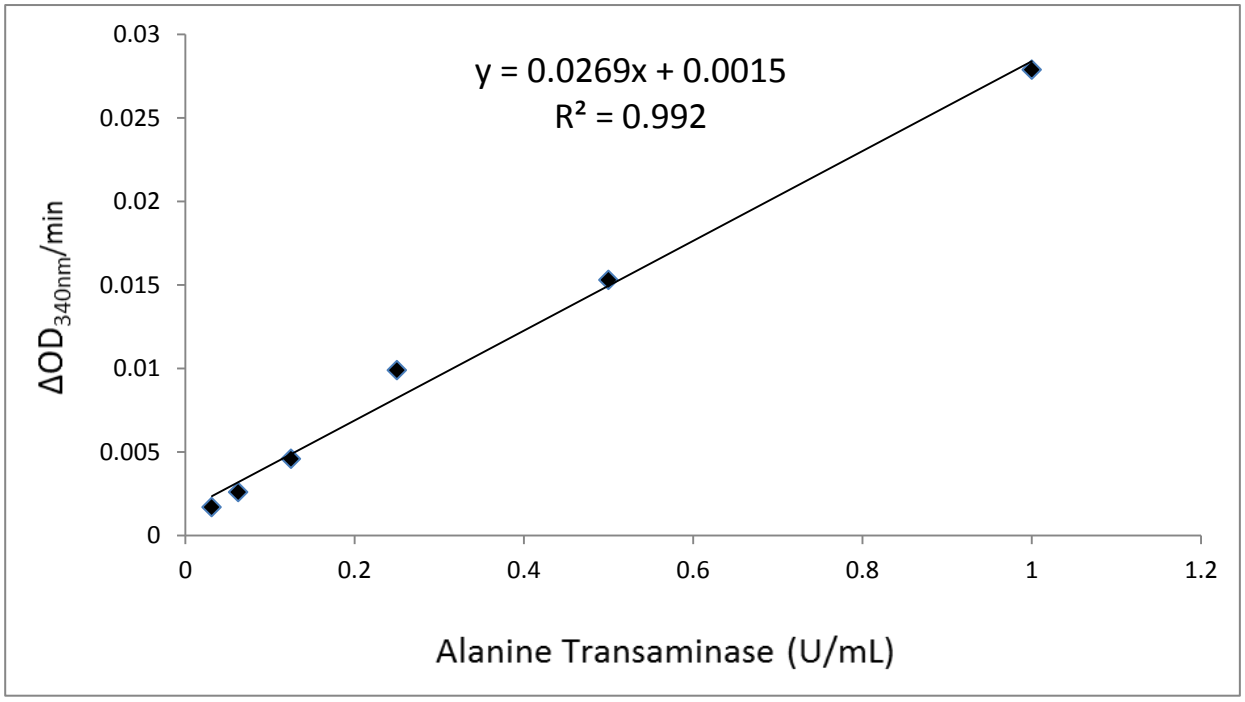

Figure2. A standard curve of ΔOD_{340nm}/min vs alanine transaminase activity, wherein theΔOD_{340nm}/min is calculated as the slope of the standard curves shown in Figure 1.