Xanthine Oxidase Assay (XO)

Cat. No. 8458
100 Tests in 96-well plate

Introduction

Xanthine Oxidase (XO) located predominantly in the liver and intestine in mammalian tissues and catalyzes the hydroxylation of hypoxanthine to xanthine and then to uric acid and hydrogen peroxide. XO activity is normally very low in blood and liver injury can result in the release of XO into blood. XO activity or expression can be up-regulated in gout and cardiovascular disease. This colorimetric assay is based on XO-catalyzed oxidation of xanthine, in which the formed hydrogen peroxide is catalyzed by peroxidase and reacts with 4-aminoantipyrine to form the product dye. The color intensity of the reaction product at 550nm is directly proportional to XO activity in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8458a	1	Assay buffer	10 mL	4°C
8458b	1	Xanthine Oxidase standard	0.2 mL	-20°C
8458c	1	Xanthine	2.0 mL	-20°C
8458d	1	Substrate mix	1.6 mL	-20°C
8458e	1	Enzyme mix	0.1 mL	-20°C

Product Use

The Xanthine Oxidase Assay kit measures the xanthine oxidase level of different types of samples, such as serum, plasma, tissues. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Serially diluted xanthine oxidase solutions with concentrations ranging from 7.81 to 125 mU/mL are measured with the ScienCell™ Xanthine Oxidase Assay kit. The increase in OD_{550nm} is monitored as a function of time (Figure 1) and the resulting standard curve of ΔOD_{550nm}/min vs xanthine oxidase activity are plotted (Figure 2). A positive linear relationship between ΔOD_{550nm}/min & xanthine oxidase activity can be observed.

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of xanthine oxidase standard

1. Add 10 µL of xanthine oxidase standard (8458b) to 30 µL of assay buffer (8458a) to make a 40 µL solution of 250 mU/mL xanthine oxidase.
2. Obtain 6 test tubes, add 25 µL of assay buffer into each tube and label them #1 through #6.
3. Add 25 µL xanthine oxidase into tube #1 and mix well to get the 125 mU/mL xanthine oxidase standard.
4. Transfer 25 µL of the 125 mU/mL xanthine oxidase standard from tube #1 to tube #2 and mix well to get the 62.5 mU/mL xanthine oxidase standard.
5. Repeat step 4 for tubes #3-5 to serially dilute the xanthine oxidase standards. Do not add any xanthine oxidase to tube #6, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each xanthine oxidase standard by aliquoting 10 µL/well of each xanthine oxidase standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6
A	125 mU/mL	62.5 mU/mL	31.25 mU/mL	15.62 mU/mL	7.81 mU/mL	blank
B	125 mU/mL	62.5 mU/mL	31.25 mU/mL	15.62 mU/mL	7.81 mU/mL	blank

B. Preparation of test samples

1. Tissues can be homogenized in 4 volumes of the assay buffer (8458a). 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 53 µL assay buffer (8458a), 20 µL xanthine (8458c), 16 µL substrate mix (8458d) and 1 µL enzyme mix (8458e).
2. Initiate the reaction by adding 90 µL of working reagent mix into each well of the 96-well plate containing xanthine standard, samples and blank.
3. Follow the increases in OD_{550nm} using a plate reader and start recording OD_{550nm} over a 20 minutes interval, collecting data every 5 minutes.

D. Calculations

1. Subtract the measured OD_{550nm} at different reaction time from the initial OD_{550nm} to obtain the corresponding ΔOD_{550nm} for each sample and xanthine oxidase standard at different reaction time. Average the value of ΔOD_{550nm} of replicate wells.
2. Based on the ΔOD_{550nm} of the xanthine oxidase standard solutions, plotting the absorbance at ΔOD_{550nm} as a

function of reaction time (Figure 1) in which ΔOD_{550nm}/min is calculated.

3. Plot a standard curve of ΔOD_{550nm}/min vs xanthine oxidase activity (Figure 2).
4. Calculate the xanthine oxidase activity of test samples based on the standard curve.

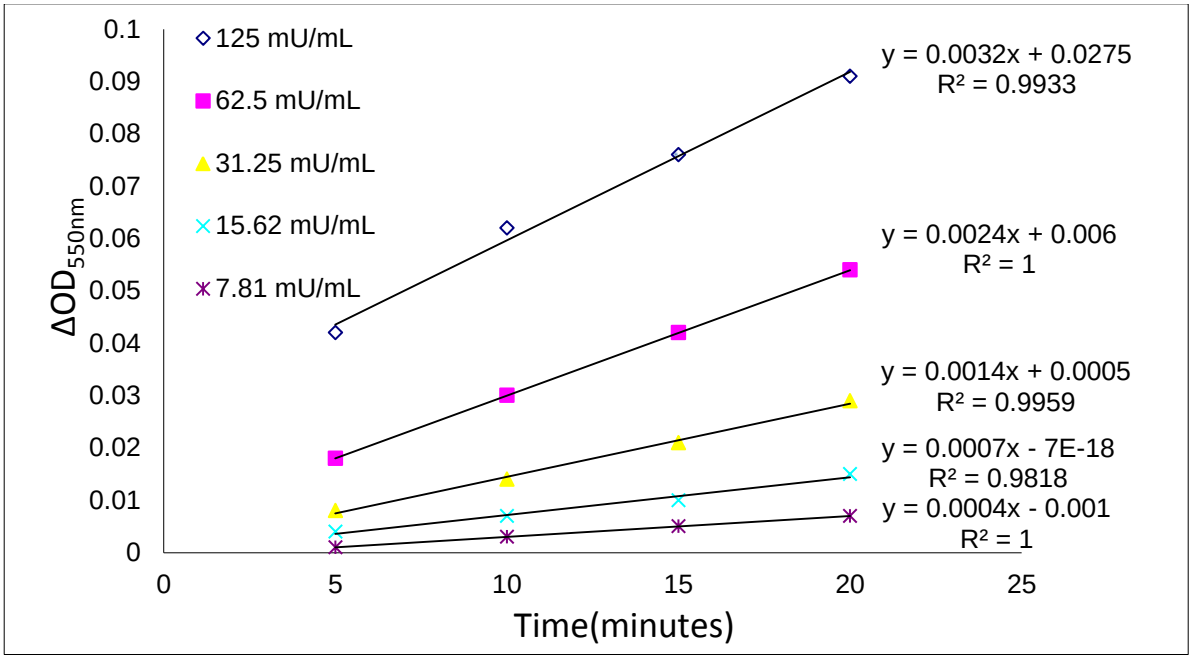

Figure1. Standard curves of ΔOD_{550nm} vs reaction time for xanthine oxidase solution with different activity.

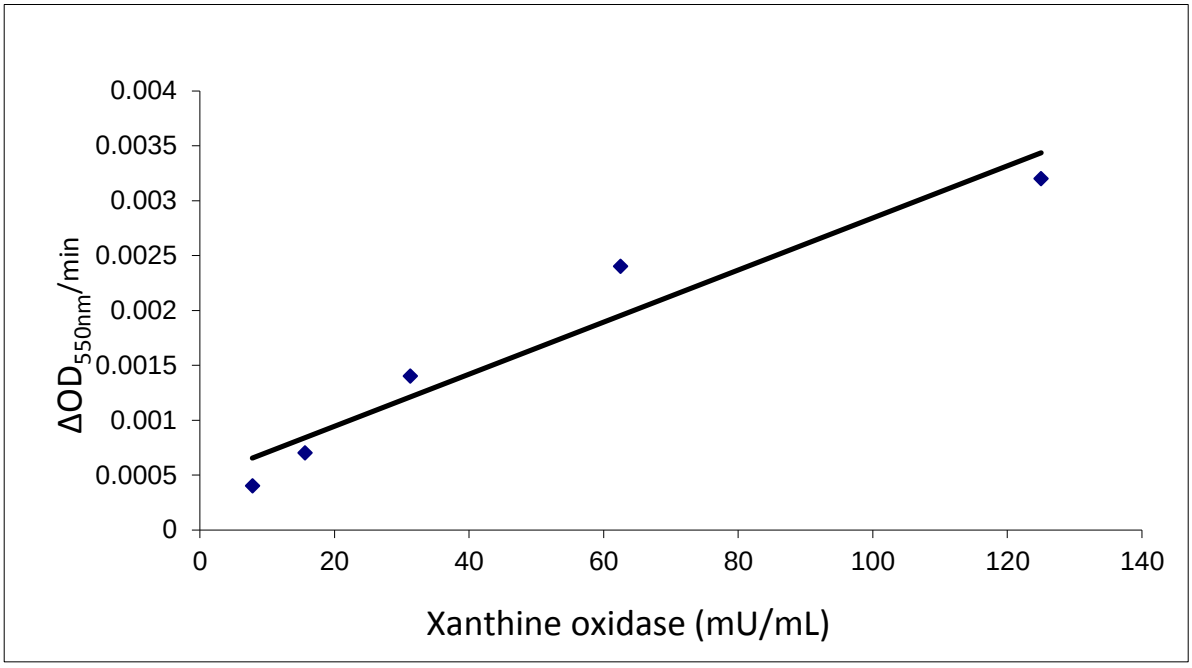

Figure2. A standard curves of ΔOD_{550nm}/min vs xanthine oxidase activity, wherein theΔOD_{550nm}/min is calculated as the slope of the standard curves shown in Figure 1.