Alcohol Dehydrogenase Assay (ADH)

Cat. No. 8568
100 Tests in 96-well plate

Introduction

Alcohol dehydrogenase (ADH) comprises a family of enzymes that catalyzes the conversion of alcohol to aldehydes in many organisms. ADH plays an important role in alcohol detoxification and leads to the generation of carcinogenic acetaldehyde which can be further converted into acetic acid by aldehyde dehydrogenase. The measurement of ADH activity in serum can be a sensitive indicator of hepatic injury and hepatitisis. This colorimetric assay is based on alcohol dehydrogenase catalyzed oxidation of ethanol, in which the formed NADH can convert a nearly colorless probe to an intensely colored product, which exhibits maximum absorbance at 440nm, which is proportional to the amount of ADH in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8568a	1	Assay buffer	25 mL	4°C
8568b	1	ADH positive control	20 µL	-80°C
8568c	1	Developer (10X)	0.1 mL	-20°C
8568d	1	NAD	0.5 mL	-20°C
8568e	1	WST	3.91 mg	-20°C
8568f	1	Substrate	1.0 mL	-20°C

Product Use

The ADH Assay kit measures the ADH activity of different types of samples, such as tissue lysate, cell lysate and plasma. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Diluted ADH positive control is measured with the ADH Assay kit after different times of reaction (Figure 1 and 2). The detection limitation range is the absorbance increase of 0.01 to 0.6 per minute.

Shipping

Shipped on dry ice.

Reagents and Positive Control Preparation

1. Diluted ADH positive control: Add 1 µl of ADH positive control into 19 µl assay buffer (8568a). Prepare diluted ADH positive control to a final volume of 10 µL/well on the 96-well flat bottom plate.
2. Developer solution (1X): dilute developer (10X) (8568c) in assay buffer (8568a) (1:10).
3. WST solution: reconstitute each vial of WST with 0.6 mL assay buffer (8568a). Vortex briefly and keep in the dark at -20°C until use. For longer storage, we suggest that you aliquot and store the reconstituted WST solution at -20°C, avoid repeated freeze/thaw cycles.

Procedure (96-well plate)

A. Preparation of test samples and blank

1. Cells or tissues can be homogenized in 4 volumes of the assay buffer (8568a). 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 serially diluted to make sure the readings are within the detection limitation range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.
3. Prepare a blank by adding 10 µL assay buffer (8568a) into one well on the 96-well flat bottom plate.

B. Working reagent preparation and measurements

1. Prepare appropriate volume of ADH assay working reagent based on the number of samples to be measured. For each well of reaction, prepare working reagent by mixing 60 µL assay buffer (8568a), 10 µL developer solution (1X), 5 µL NAD (8568d) and 5 µL WST solution, 10 µL substrate(8568f).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing diluted ADH positive control, samples and blank, mix well immediately and start recording OD_{440nm} over 3 minuteintervals, collecting data every 0.5 min. Figure 1 shows data of ADH positive control.

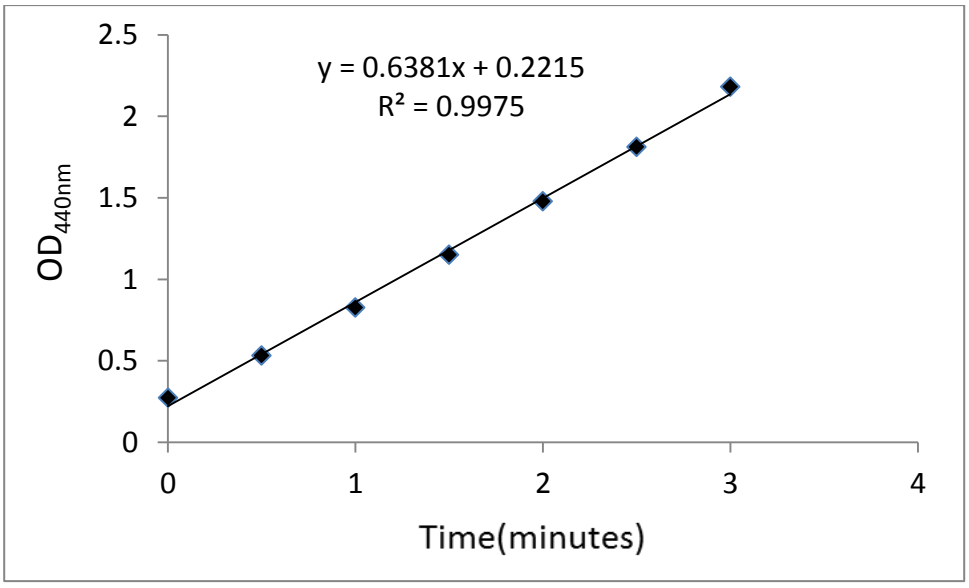

Figure.1 Absorbance change of diluted ADH positive control at 440nm.

C. Calculations

1. Determine the change in absorbance ΔOD_{440nm}/min by plotting the absorbance value at ΔOD_{440nm} as a function of reaction time to obtain the slope of the linear portion of the curve. As shown in Figure.2.

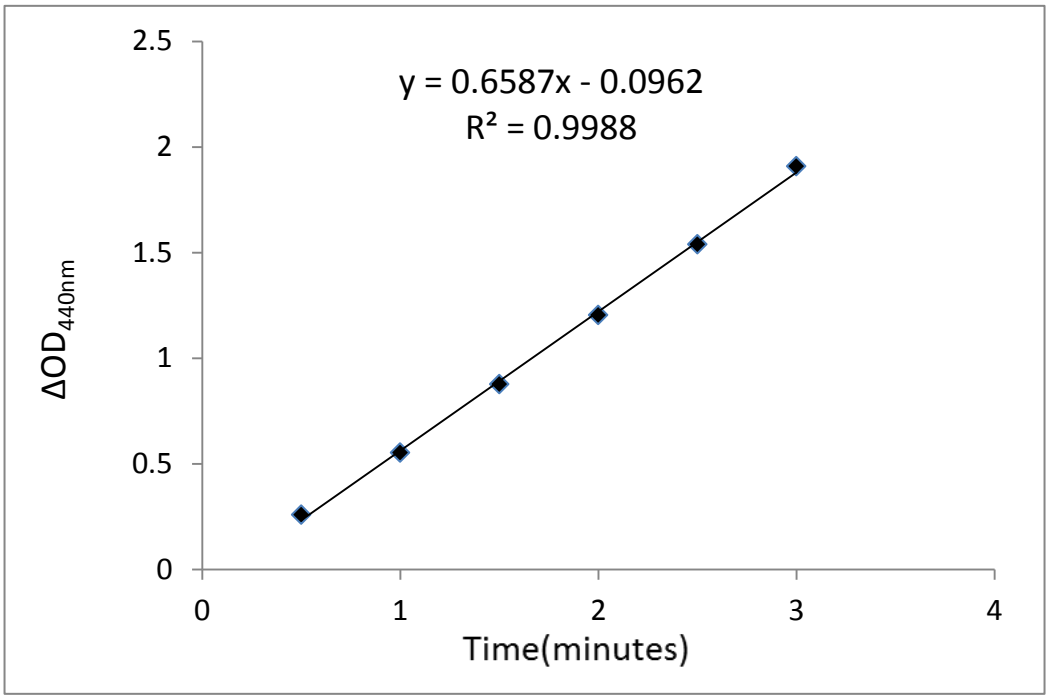

Figure.2 The change in absorbance ΔOD_{440nm} of diluted ADH positive control during the time at 440nm.

2. Calculate the ADH activity using the following formula:

$$\text{ADH (U/ml)} = \frac{\Delta OD_{440nm}/\text{min} \times 100\ \mu l}{11.53\ mM^{-1} \times 10\ \mu l} \times \text{sample dilution}$$

Note: The actual extinction coefficient of the formed WST-1 formazan at 440nm is 37 $mM^{-1}cm^{-1}$. This value has been adjusted for the path length of the solution in the 96-well plate.

Unit definition: One unit would make 1.0 µmol of WST-1 to WST-1 formazan per minute at pH 8.5 at 25 ºC

3. Use the formula to calculate ADH positive control activity:

$$\text{ADH (U/ml)} = \frac{0.6587 \times 100\ \mu l}{11.53 mM^{-1} \times 10\ \mu l} \times 20 = 11.42\ \text{(U/ml)}$$