Aldehyde Dehydrogenase Assay (ALDH)

Cat. No. 8578

100 Tests in 96-well plate

Introduction

Aldehyde dehydrogenase (ALDH) is a family of enzymes that catalyze the conversion of various aldehydes to their corresponding carboxyolic acids. ALDH plays an important role in alcohol metabolism, converting acetaldehyde, which has carcinogenic, cytotoxicity, and mutagenicity affects, into acetic acid. The change in ALDH activity and expression levels can be used as a marker for cancer, cancer stem cells, and Parkinson's disease. This colorimetric assay is based on aldehyde dehydrogenase-catalyzed oxidation of aldehyde, where the resulting NADH can then convert a nearly colorless probe to a colored product; the intensity of the colored product is proportional to the amount of ALDH in the sample, exhibiting maximum absorbance at 440nm.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8578a	1	Assay buffer	25 mL	4°C
8578b	1	ALDH positive control	20 µL	-80°C
8578c	1	Developer (10X)	0.1 mL	-20°C
8578d	1	NAD	0.5 mL	-20°C
8578e	1	WST	3.91 mg	-20°C
8578f	1	Cofactor	0.5 mL	4°C
8578g	1	Substrate	0.5 mL	-20°C

Product Use

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

Quality Control

Diluted ALDH positive control is measured with the ALDH Assay kit after various reaction times (Figure 1 and 2). The detection range has a limitation of an absorbance increase of 0.01 to 0.5 per minute.

Shipping

Shipped on dry ice.

Reagents and Positive Control Preparation

1. Diluted ALDH positive control: Add 1 µl of ALDH positive control into 79 µl assay buffer (8578a). Prepare diluted ALDH positive control to a final volume of 10 µL/well in a 96-well flat bottom plate.
2. Developer solution (1X): dilute developer (10X) (8578c) in assay buffer (8578a) (1:10).
3. WST solution: reconstitute each vial of WST with 0.6 mL assay buffer (8578a). 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 (8578a). 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 in a 96-well flat bottom plate.
3. Prepare a blank by adding 10 µL assay buffer (8578a) into one well of a 96-well flat bottom plate.

B. Working reagent preparation and measurements

1. Prepare appropriate volume of ALDH 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 (8578a), 10 µL developer solution (1X), 5 µL NAD (8578d), 5 µL WST solution, 5 µL cofactor (8578f), and 5 µL substrate (8578g). *Note: the substrate is carcinogenic and should be used in a fume hood.*
2. Add 90 µL of working reagent mix into each well of a 96-well plate containing the diluted ALDH positive control, samples, and blank. Immediately mix well and start recording OD_{440nm} over 4 minute intervals, collecting data every 0.5 min. Figure 1 shows the data of ALDH positive control.

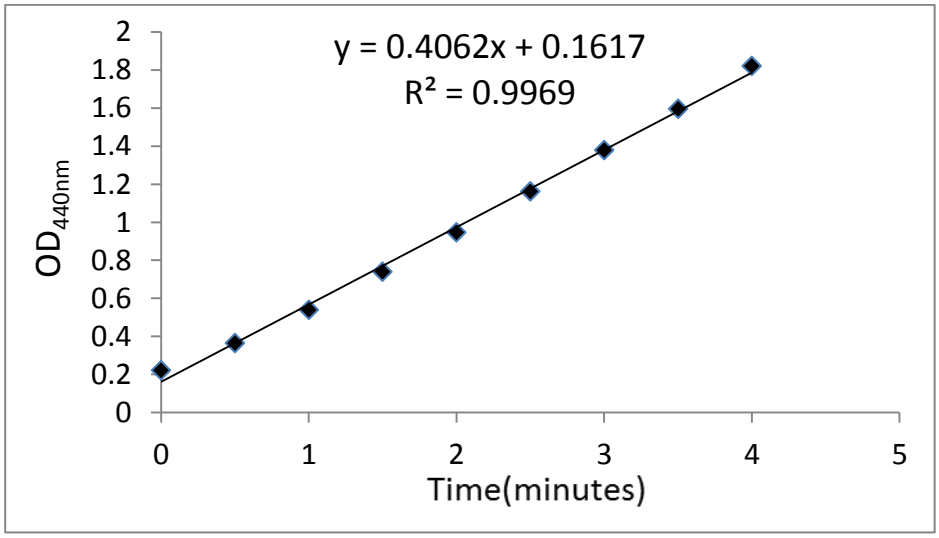

Figure.1 Absorbance change of diluted ALDH positive control at 440nm.

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.

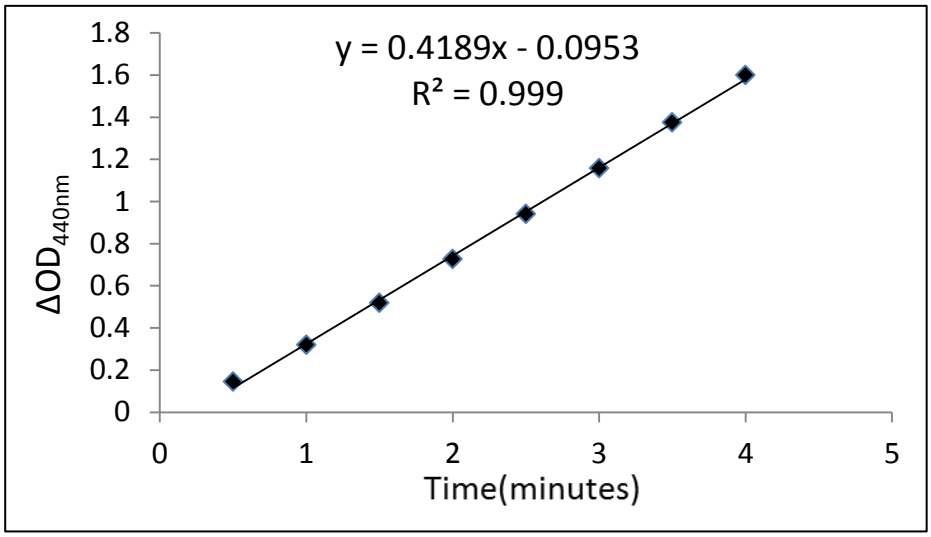

Figure.2 The change in absorbance ΔOD_{440nm} of diluted ALDH positive control during the indicated time at 440nm.

2. Calculate ALDH activity using the following formula:

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

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 a 96-well plate.

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

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

$$\text{ALDH (U/ml)} = \frac{0.4189 \times 100\ \mu l}{11.53 mM^{-1} \times 10\ \mu l} \times 80 = 29.065\ \text{(U/ml)}$$