Malate Dehydrogenase Assay (MDH)

Cat. No. 8638

100 Tests in 96-well plate

Introduction

Malate dehydrogenase (MDH2) catalyzes the last reaction of the TCA or Krebs cycle, converting malate and NAD+ to oxaloacetate and NADH in the mitochondria. In reversing the reaction, MDH is also involved in gluconeogenesis, allowing oxaloacetate/malate to leave the mitochondria. Once in the cytosol, the malate is oxidized back to oxaloacetate by cytosolic MDH or MDH1, which is followed by conversion of oxaloacetate to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (PEPCK). The interconversion between malate and oxaloacetate also constitutes the essential steps of the malate-aspartate shuttle. This colorimetric assay is based on malate dehydrogenase-catalyzed oxidation of malate, 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 MDH in the sample, exhibiting maximum absorbance at 440nm.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8638a	1	Assay buffer	25 mL	4°C
8638b	1	MDH positive control	20 µL	-20°C
8638c	1	Developer (10X)	0.1 mL	-20°C
8638d	1	NAD	0.5 mL	-20°C
8638e	1	WST	3.91 mg	-20°C
8638f	1	Cofactor	0.5 mL	4°C
8638g	1	Substrate	0.5 mL	4°C

Product Use

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

Quality Control

Diluted MDH positive control is measured with the MDH Assay kit after various reaction times (Figure 1 and 2). The linear range of detection is 0.15 to 1.0 mU/ml in a 96-well plate assay.

Shipping

Shipped on dry ice.

Reagents and Positive Control Preparation

1. Diluted MDH positive control: Add 1 µl of MDH positive control into 39 µl assay buffer (8638a). Prepare diluted MDH positive control to a final volume of 10 µL/well in a 96-well flat bottom plate.
2. Developer solution (1X): dilute developer (10X) (8638c) in assay buffer (8638a) (1:10).
3. WST solution: reconstitute each vial of WST with 0.6 mL assay buffer (8638a). 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 (8638a). 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 (8638a) into one well of a 96-well flat bottom plate.

B. Working reagent preparation and measurements

1. Prepare appropriate volume of MDH 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 (8638a), 10 µL developer solution (1X), 5 µL NAD (8638d), 5 µL WST solution, 5 µL cofactor (8638f), and 5 µL substrate (8638g).
2. Add 90 µL of working reagent mix into each well of a 96-well plate containing the diluted MDH positive control, samples, and blank. Immediately mix well and start recording OD_{440nm} over 40 minute intervals, collecting data every 5 min. Figure 1 shows the data of diluted MDH positive control.

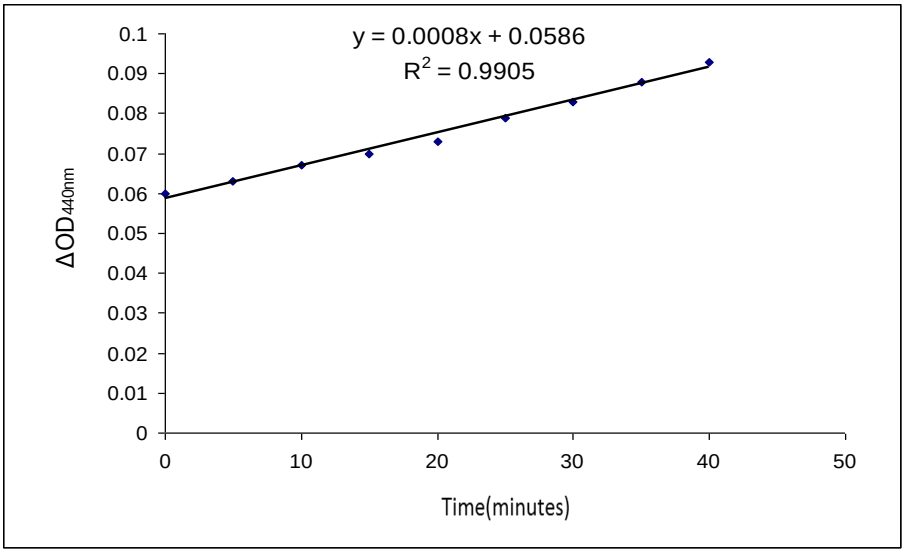

Figure.1 Absorbance change of diluted MDH positive control at 440nm.

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.

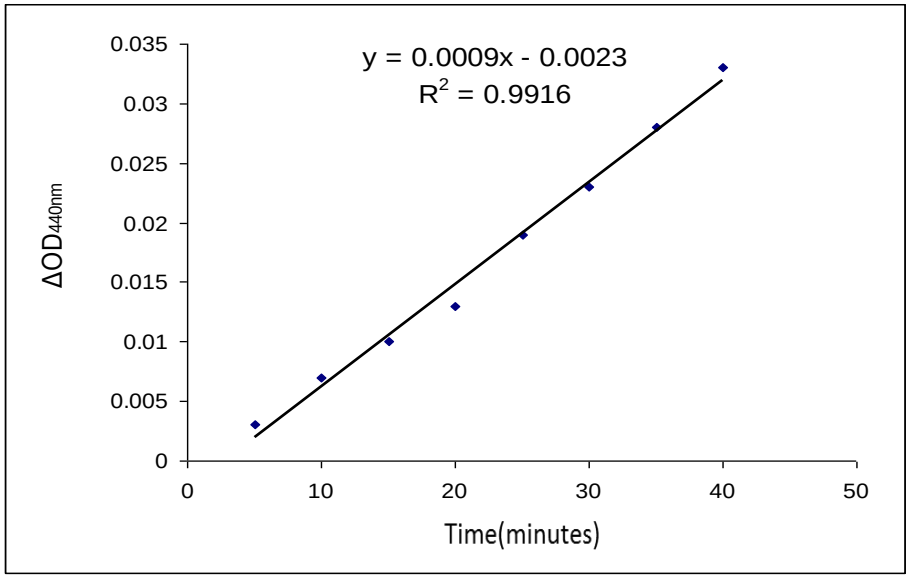

Figure.2 The change in absorbance ΔOD_{440nm} of diluted MDH positive control during the indicated time at 440nm.

2. Calculate MDH activity using the following formula:

$$\text{MDH (U/ml)} = \frac{(T_{40} - T_0) \times 100\ \mu l}{40 \times 11.53\ mM^{-1} \times 10\ \mu l} \times \text{sample dilution}$$

Note:

a. T_{40} and T_0 are absorbance readings of the diluted MDH positive control at 40 minutes and 0 minutes, respectively. 40 is the enzyme reaction time.

b. 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 10.0 at 25 ºC

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

$$\text{MDH (U/ml)} = \frac{0.033 \times 100\ \mu l}{40 \times 11.53\ mM^{-1} \times 10\ \mu l} \times 40 = 0.0286\ \text{(U/ml)}$$