Malate Assay (Mal)

Cat. No. CB019
(100 Tests in 96-well plate)

Introduction

L-Malate (Mal) is a tricarboxylic acid cycle intermediate and a critical component of the malate-aspartate shuttle. Malate also improve muscle performance and act as a metal chelator. In plants, malate is a source of CO_2 for the Calvin Cycle in the C4 carbon fixation process. Malate is frequently used as an additive in the food and pharmaceutical industries. 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 malate in the sample, exhibiting maximum absorbance at 440nm.

Kit Components

Assay buffer, 25 mL
Malate standard, 0.5 mL
Developer (10X), 0.1 mL
NAD, 0.5 mL
WST, 3.91 mg
Cofactor, 0.5 mL
Enzyme, 0.1 mL

Reagents preparation

1. Developer solution (1X): dilute developer (10X) in assay buffer (1:10).
2. WST solution: reconstitute each vial of WST with 0.6 mL assay buffer. 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.
3. Diluted malate standard: Add 1 µL malate standard into 99 µL assay buffer.

Procedure (96-well plate)

A. Preparation of malate standard

1. Add 25 µL of diluted malate standard to 100 µL of assay buffer to make a 125 µL solution of 0.2 mM malate.
2. Obtain 6 test tubes, add 110 µL of assay buffer into each tube and label them #1 through #6.
3. Add 110 µL of the 0.2 mM malate solution into tube #1 and mix well to obtain the 0.1 mM malate standard.
4. Transfer 110 µL of the 0.1 mM malate standard from tube #1 to tube #2 and mix well to obtain the 0.05 mM malate standard.
5. Repeat step 4 for tubes #3-5 to serially dilute the malate standards. Do not add any malate to tube #6, which serves as a blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A and B) of each malate standard by aliquoting 50 µL/well of each malate standard into duplicate wells of the 96-well test plate, following the plate format below:

	#1	#2	#3	#4	#5	#6
A	0.1 mM	0.05 mM	0.025 mM	0.0125 mM	0.00625 mM	blank
B	0.1 mM	0.05 mM	0.025 mM	0.0125 mM	0.00625 mM	blank

B. Preparation of test samples and blank

1. Cells or tissues can be homogenized in 4 volumes of the assay buffer. 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 into one well of a 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. Prepare appropriate volume of malate 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, 10 µL developer solution (1X), 5 µL NAD, 5 µL WST solution, 5 µL cofactor, and 5 µL enzyme.
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing malate standard, samples and blank. Measure the plate immediately at 440 nm with an ELISA plate reader to get A_0.
3. Incubate for 60 minutes at room temperature in dark. Read the absorbance at 440 nm with an ELISA plate reader to get A_{60}.

D. Calculations

1. Subtract the A_0 value from the A_{60} obtained with all other standard and samples to get ΔA_{60} value. Subtract the A_{60} value of the blank from the ΔA_{60} value obtained with all other standard and
2. samples to get $\Delta\Delta A_{60}$ value.
3. Based on the calibrated $\Delta\Delta A_{60}$ of the malate standard, make a standard curve by plotting $\Delta\Delta A_{60}$ as a function of malate concentration (see Figure 1 for a typical standard curve). Determine the equation and R^2 value of the resulting trend line.
4. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the malate concentration of test samples as follows:

$$[\text{Malate}] = \frac{A_{60} - B}{A}$$

Usage

Malate Assay is used to coat cell culture vessels *in vitro*. Malate Assay is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.