EnzyChrom™ Malate Assay Kit (EMAL-100)

Quantitative Colorimetric L-Malate (L-Malic Acid) Determination

DESCRIPTION

L-MALIC ACID, or L-malate, is a dicarboxylic acid that is made by all living organisms and plays an important role in the Calvin and Krebs Cycle. It is a source of CO_2 for the Calvin cycle in plants and is also an intermediate that forms from fumarate in the Krebs Cycle. Malate is frequently used in food and beverage industries as an additive in products such as wine, beer, candies, etc.

BioAssay Systems' L-malate assay kit is based on malate dehydrogenase catalyzed oxidation of malate in which the formed NADH reduces a formazan (MTT) reagent. The intensity of the product color, measured at 565 nm is proportional to the malate concentration in the sample.

KEY FEATURES

Fast and sensitive. Use of 20µL sample. Linear detection range 0.02 to 2 mM L-malate in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the optical density at time 15 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: malate in food, juice, beverage and other agricultural products.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
NAD/MTT:	1 mL	**Enzyme B:**	120 µL
Standard:	1.0 mL 20mM L-Malate		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation: clear and slightly colored samples can be assayed directly.

Solid samples (food, fruits etc) can be homogenized in water followed by filtration or centrifugation (e.g. 5 min 14,000 rpm). *Beverage samples* can be assayed directly. Prior to assay, check the pH of the sample. If the pH is not between 7 and 8, adjust the sample pH to 7-8 with NaOH or HCl. *S*amples containing carbon dioxide should be degassed by gentle stirring prior assay. No dilution necessary in general.

It is prudent to test several dilutions to determine an optimal dilution factor *n*.

All samples can be stored at –20 to –80°C for at least one month.

1. *Standards and Samples*. Equilibrate all components to room temperature. Prepare 500 µL 2.0 mM L-Malate Premix by mixing 50 µL 20 mM Standard and 450 µL distilled water. Dilute standard as follows.

No	Premix + H_2O	Vol (µL)	L-Malate (mM)
1	100 µL + 0 µL	100	2.0
2	60 µL + 40 µL	100	1.2
3	30 µL + 70 µL	100	0.6
4	0 µL + 100 µL	100	0

Transfer 20 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 20 µL of each sample into two separate wells, one serving as a sample blank well (OD_{BLANK}) and one as a sample well (OD_{SAMPLE}).

2. Prepare sufficient Working Reagent (WR) by mixing for each standard and sample well, 74 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B and 8 µL NAD/MTT. Prepare blank Working Reagent (BWR) by mixing for each sample blank well, 74 µL Assay Buffer, 1 µL Enzyme B and 8 µL NAD/MTT (i.e. no Enzyme A). Fresh reconstitution of the WRs is recommended.

   Add 80 µL WR to the *four Standards* and the *Sample Wells*. Add 80 µL BWR to the *Sample Blank Wells*. Tap plate to mix briefly and thoroughly. Incubate 15 minutes at room temperature.

3. Read optical density at 565nm (520-600nm).

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the L-malate concentration of Sample,

$$[\text{L-Malate}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \ (\mu M)$$

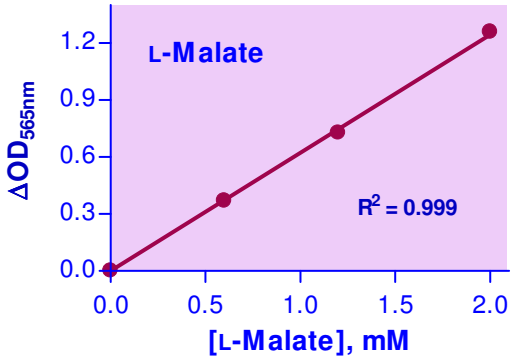
OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

Note: if the sample OD value is higher than OD for 2 mM L-malate standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM L-malate equals 13.3 mg/dL, 0.018% or 133 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate.



Standard Curve in 96-well plate assay in water.

LITERATURE

1. Shapiro, F. and Ianikove, N (2011). Rapid and accurate determination of malate, citrate, pyruvate and oxaloacetate by enzymatic reactions coupled to formation of a fluorochromophore: Application in colorful juices and fermentable food (yogurt, wine) analysis. Elsevier Science B.V., Amsterdam; 129, 2; 608-613.

2. Mollering, H (1985). L-Malate. In: Methods of Enzymatic Analysis (Bergmeyer, h. U., ed.), 3rd ed., Vol,VII, pp. 39-47, VCH Publishers (UK), Cambridge, UK.

3. M.F.S. Peres et al. (2008). Colorimetric Enzymatic Assay of L-Malic Acid, Food Technol. Biotechnol. 46 (2) 229-233.