Technical Bulletin

Malate Assay Kit

Catalogue number MAK511

Product 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.

The 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.

The linear detection range of the kit is 0.02 to 2 millimolar (mM) L-Malate. The kit is suitable for determination of malate in food, juice, beverage and other agricultural products.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK511A
- Enzyme A 120 µL
  Catalogue Number MAK511B
- Enzyme B 120 µL
  Catalogue Number MAK511C
- NAD/MTT 1 mL
  Catalogue Number MAK511D
- Standard (20mM L-Malate) 1 mL
  Catalogue Number MAK511E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example multichannel pipettor).
- Multiwell plate reader.
- Clear flat-bottom 96-well plates and 96-well plate absorbance reader for procedure using 96-well plate. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

Sample Preparation

1. Clear and slightly colored Samples can be assayed directly.
2. For Solid Samples, homogenize solid samples such as food or fruits in water. Filter or centrifuge at 14,000 rpm for 5 minutes.
3. For Beverage samples, assay can be performed directly. Prior to assay, check the pH of the Sample. If necessary, adjust the Sample pH to 7-8 with NaOH or HCl.
4. Samples containing carbon dioxide should be degassed by gentle stirring prior assay. No dilution necessary in general.
5. Transfer 20 µL of the Sample into two separate wells. One well serves as Sample Blank and one well as Sample well.

Colorimetric Standard Curve Preparation

1. Prepare 500 µL of 2 mM L-Malate Standard by mixing 50 µL of 20 mM Standard and 450 µL purified water. Dilute Standards as described in the Table 1

Table 1.
Preparation of Colorimetric L-Malate Standard

No.	2 mM L-Malate Standard	Purified Water	L-Malate (mM)
1	100 µL	0 µL	2.0
2	60 µL	40 µL	1.2
3	30 µL	70 µL	0.6
4	0 µL	100 µL	0

2. Transfer 20 µL Standards into separate wells of a clear, flat-bottom 96-well plate.

Working Reagents

1. Mix enough reagents for the number of assays to be performed. Prepare Working Reagent and Blank Working Reagent according to Table 2.
2. For each Standard and Sample well, prepare 84 µL of Working Reagent and 83 µL of blank Working Reagent for each sample blank well.

Note: Fresh reconstitution of the working reagents is recommended.

Table 2.
Preparation of Working Reagents for 96-well plate assay

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	74 µL	74 µL
MTT/NAD	8 µL	8 µL
Enzyme A	1 µL	-
Enzyme B	1 µL	1 µL

3. Add 80 µL of Working Reagent to Standards and Sample wells or Blank Working Reagent to corresponding Sample Blank wells. Tap plate to mix briefly and thoroughly.

Measurement

1. Incubate 15 minutes at room temperature.
2. Measure the optical density at 565 nm.

Results

1. Calculate ΔOD by subtracting the reading of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD against the standard concentrations and determine the slope of the standard curve.
3. Calculate the L-malate concentration of samples using the below equation:

$$[\text{L-Malate}] = \frac{R_{Sample} - R_{Blank}}{\text{Slope}} \times DF$$

Where:

R_{Sample} = OD reading of Sample

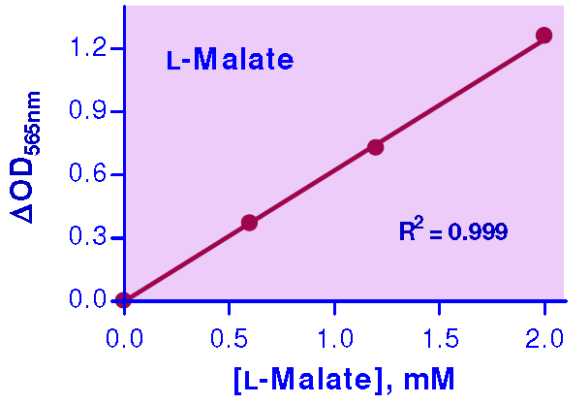
R_{Blank} = OD reading of Sample Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated concentration of Sample is higher than 2 mM L-malate Standard, dilute the Sample in water and repeat the assay. Multiply the results by the dilution factor (DF).

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

Figure 1.
Typical Colorimetric L-Malate Standard Curve



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak511pis Rev 10/23