Product Manual

Malate Assay Kit (Fluorometric)

Catalog Number

MET-5120 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Malate, the anion salt of malic acid, is a dicarboxylic acid that is found in all living organisms. Malate plays an important role in biochemistry. In the process of C4 carbon fixation, malate provides CO2 in the Calvin cycle. In the Krebs cycle, malate is created by the addition of an -OH group to fumarate. Malate can additionally be formed from pyruvate by anaplerotic reactions. Malate is also created in the guard cells of plant leaves by phosphoenolpyruvate carboxylation.

Malate is often added to various foods and therefore quantitation of malate is important in various industries such as fruit, wine, beer, and cheese manufacturing. In the fruit industry, levels of malic acid have been found to be higher in organic crops as opposed to other standard methods of agriculture.

Assay Principle

Cell Biolabs' Malate Acid Assay Kit (Fluorometric) measures the malate within beverages, food, serum, plasma, and cell or tissue lysate samples. The assay is based on an enzyme driven reaction: when malate incubated in the presence of Malic Acid dehydrogenase (MDH) and NAD^+, NAD^+ is converted to its reduced form NADH. Diaphorase then uses NADH to reduce resazurin to resorufin which is then detected fluorometrically (Figure 1).

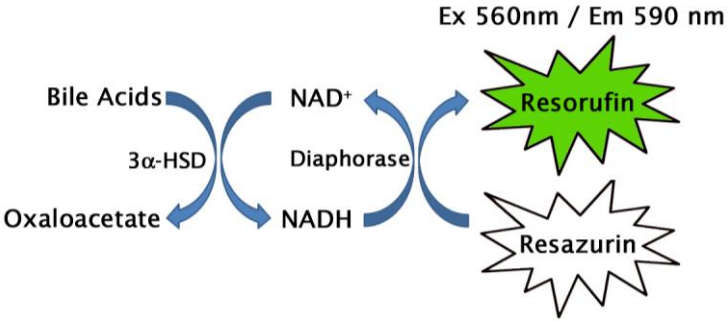

Figure 1. Assay Principle.

Related Products

1. MET-5030: NAD+/NADH Assay Kit (Fluorometric)
2. MET-5005: Total Bile Acid Assay Kit (Fluorometric)
3. STA-619: Free Fatty Acid Assay Kit (Fluorometric)
4. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
5. MET-5137: D-Amino Acid Assay Kit (Fluorometric)

Kit Components

1. L-Malate Standard (Part No. 51191C): One 100 µL vial at 200 mM.
2. 10X Assay Buffer (Part No. 51192A): One 30 mL bottle.
3. Assay Reagent (Part No. 51201D): Three 1.7 mL vials containing NAD+, diaphorase, and resazurin.
4. L-Malic Acid Dehydrogenase (100X) (Part No. 51202B): One 60 µL vial at 400 U/mL

Note: One unit is defined as the amount of enzyme that reduces 1 µmol of oxaloacetate and ß-NADH to L-malate per minute at 25 ºC and pH 7.5.

Materials Not Supplied

1. Distilled or deionized water
2. Standard 96-well clear microtiter plate
3. Standard 96-well fluorescence black microtiter plate

Storage

Upon receipt, store the 10X Assay Buffer and the L-Malic Acid Dehydrogenase at 4°C (DO NOT FREEZE L-Malic Dehydrogenase). Store the L-Malate Standard and the Assay Reagent at -80°C and avoid multiple freeze/thaw cycles.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at 4°C.
- Working Assay Reagent: Dilute the L-Malic Acid Dehydrogenase (100X) 1:100 into the provided Assay Reagent. For example, for 20 assays add 10 µL of L-Malic Acid Dehydrogenase (100X) to 990 µL of Assay Reagent.

Note: Scale down the described example appropriately and prepare only enough for immediate use.

Preparation of Samples

Notes: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with unknown samples.

- Liquid beverage samples such as beer, wine, juice: Samples can be assayed undiluted or diluted as necessary in deionized water.
- Solid food samples such as fruit or cheese: Samples can be processed by homogenization of 20 mg solid with 500 µL of water (at 40-50°C) for 30 minutes. Pellet the insoluble material for 10 minutes at 10000-14000xg. Recover the soluble fraction and dilute as necessary in deionized water.
- Cell culture supernatants: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Prepare the Malate standard curve in the same non-conditioned media.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris at 18000 xg for 15 minutes at 4°C. Cell lysates can be assayed undiluted or diluted as necessary in deionized water.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary in deionized water.

Preparation of Standard Curve

Prepare fresh Malate standards before use.

1. First, **dilute the stock L-Malate Standard 200 mM solution 1:10 in 1X Assay Buffer for a 20 mM Malate Solution**. (e.g., add 5 µL of the stock 200 mM L-Malate Standard to 45 µL of 1X Assay Buffer). **Vortex thoroughly**.

2. Use the 20 mM Malate Solution to prepare a series of the remaining Malate standards according to Table 1.

Standard Tubes	20 mM Malate Solution (µL)	1X Assay Buffer (µL)	Malate (µM)
1	10	490	400
2	250 of Tube #1	250	200
3	250 of Tube #2	250	100
4	250 of Tube #3	250	50
5	250 of Tube #4	250	25
6	250 of Tube #5	250	12.5
7	250 of Tube #6	250	6.25
8	0	250	0

Table 1. Preparation of Malate Standards

Assay Protocol

Each Malate standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 50 µL of the diluted malate standards or samples to the 96-well microtiter black plate.

2. Add 50 µL of Working Assay Reagent (see Preparation of Reagents section) to each well.

3. Incubate at room temperature for 45-60 minutes protected from light.

4. Read the plate at an excitation wavelength of 560 nm and an emission wavelength 590 nm using a microplate fluorometer.

Example of Results

The following figures demonstrate typical Malate Assay (Fluorometric) results. One should use the data below for reference only. This data should not be used to interpret actual results.

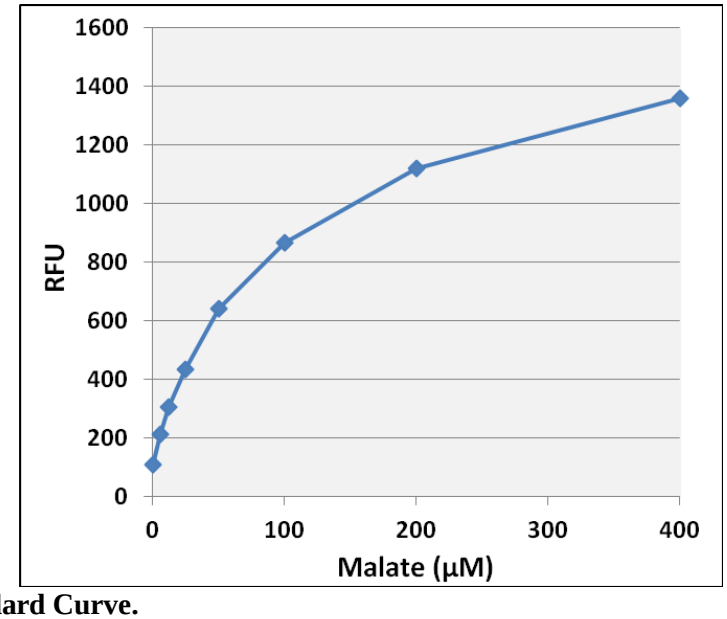

Figure 2. Malate Standard Curve.

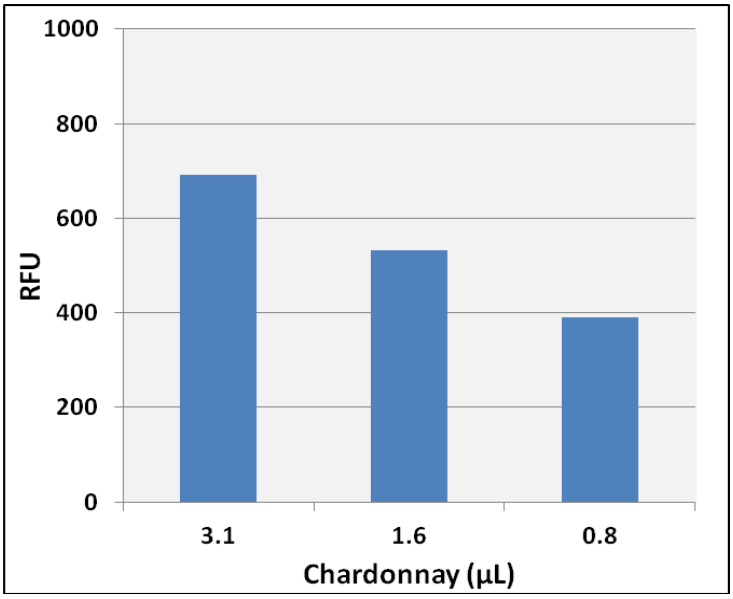

Figure 3. Detection of Malate in Chardonnay. La Crema Chardonnay Sonoma Coast 2015 Wine was assayed according to the kit protocol.

References

1. Kupina SA, Pohl CA, and Gannotti JL (1991) Am *J Enol Vitic* **42**: 1-5.
2. Klopper W.J, Angelino SAGF, Tuning B. and Vermeire HA (1986) *J. Inst. Brew*, **92**:225-228
3. Xu J, Zhai Y, Feng L, Xie T, Yao W, Shan J, and Zhang L. *J Pharm Biomed Anal*. (2019) **171**:171-179.
4. Hatch, MD. (2002). *Photosynth. Res*. **73**: 251–6.
5. Cushman, JC. (2001). *Plant Physiol*. **127**: 1439–1448.
6. Chinopoulos, C (2013). *J. Neurosci*. *Res*. **91**: 1030–43.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com