Product Manual

Malate Assay Kit (Colorimetric)

Catalog Number

MET-5119 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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

The Malate Assay Kit (Colorimetric) is a sensitive, quantitative colorimetric assay. The provided reagents are sufficient for the evaluation of 200 assays*. The unknown samples or malate standards are added to a 96 well plate followed by the Colorimetric Probe Mix containing WST-1, an electron mediator, and L-Malic Acid Dehydrogenase (MDH). During a brief incubation the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. The content of malate in the unknown samples is determined by comparison with a predetermined malate standard curve.

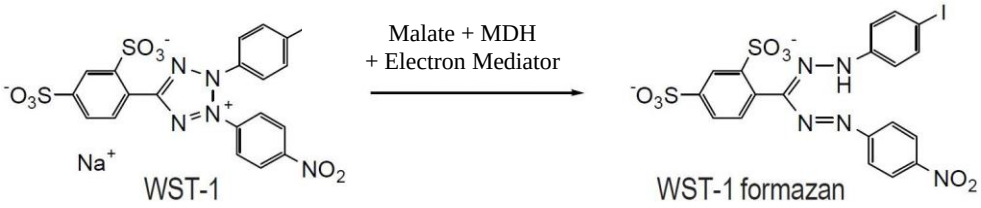

Figure 1. Assay Principle.

Note: Each sample replicate requires 2 assays, one treated with L-malic acid dehydrogenase (+MDH) and one without (-MDH). Malate levels are calculated from the difference in OD readings from the 2 wells.

Related Products

1. MET-5120: Malate Assay Kit (Fluorometric)
2. MET-5029: Pyruvate Assay Kit
3. MET-5080: Glutamate Assay Kit (Colorimetric)
4. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
5. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
6. MET-5056: Branched Chain Amino Acid Assay Kit
7. MET-5070: Glycine Assay Kit
8. MET-5073: Tyrosine Assay Kit

9. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
10. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
11. STA-341: OxiSelect™ Catalase Activity Assay Kit

Kit Components

1. 10X Colorimetric Probe (Part No. 50801C): Two 2 mL amber vials.
2. L-Malate Standard (Part No. 51191C): One 100 µL vial at 200 mM.
3. 10X Assay Buffer (Part No. 51192A): One 30 mL bottle.
4. 50X NAD+ (Part No. 50803D): One 800 µL vial.
5. L-Malic Acid Dehydrogenase (100X) (Part No. 51193B): One 400 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. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Standard 96-well clear microtiter plate
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)

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 50X NAD+ at -80°C. Store all remaining components at -20°C. The 10X Colorimetric Probe is light sensitive and must be stored accordingly. 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.
- Reaction Mix: Dilute the 10X Colorimetric Probe, the L-Malic Dehydrogenase (100X) and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 µL of 10X Colorimetric Probe, 40 µL of L-Glutamic Dehydrogenase (100X), and 80 µL of 50X NAD+ to 3.48 mL of 1X Assay Buffer.

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

- Control Mix: Dilute both the 10X Colorimetric Probe and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 µL of 10X Colorimetric Probe, and 80 µL of 50X NAD+ to 3.52 mL of 1X Assay Buffer.

  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 of solid with 500 µL of water (at 40-50°C) for 30 minutes. Pellet the insoluble material for 10 minutes at 10000-14000 xg. 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. 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). Use the 20 mM Malate Solution to prepare a series of the remaining Malate standards according to Table 1 below.

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

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with MDH (Reaction Mix) and one without the enzyme (Control Mix) to measure endogenous sample background.
2. Add 50 µL of each sample (Malate standard or unknown) into wells of a 96 well plate.
3. Add 200 µL of Reaction Mix to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 200 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate at room temperature for 60 minutes on an orbital shaker.
6. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

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

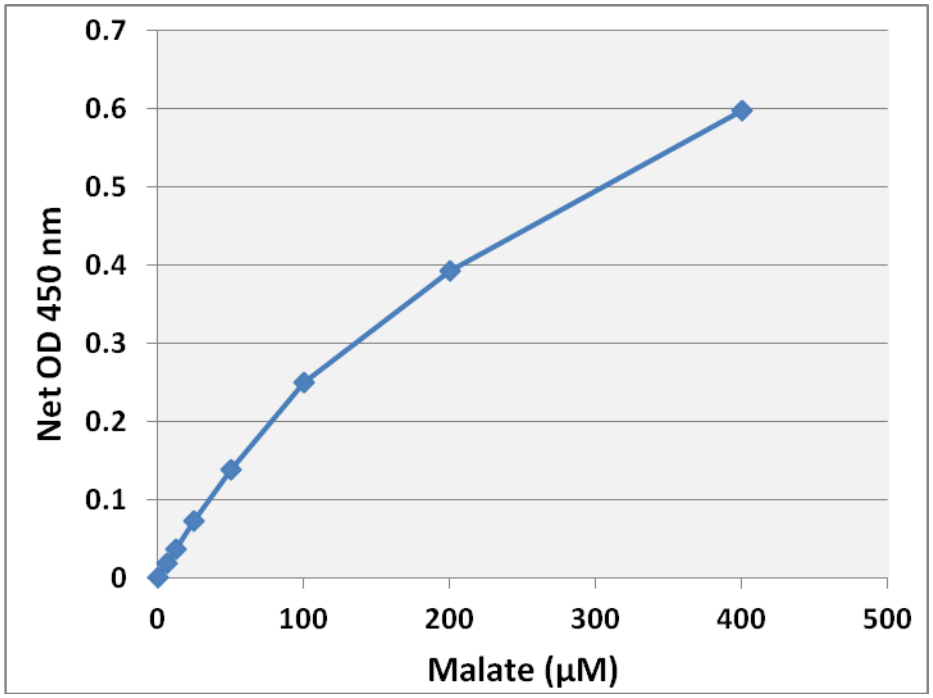

Figure 2. Malate Standard Curve.

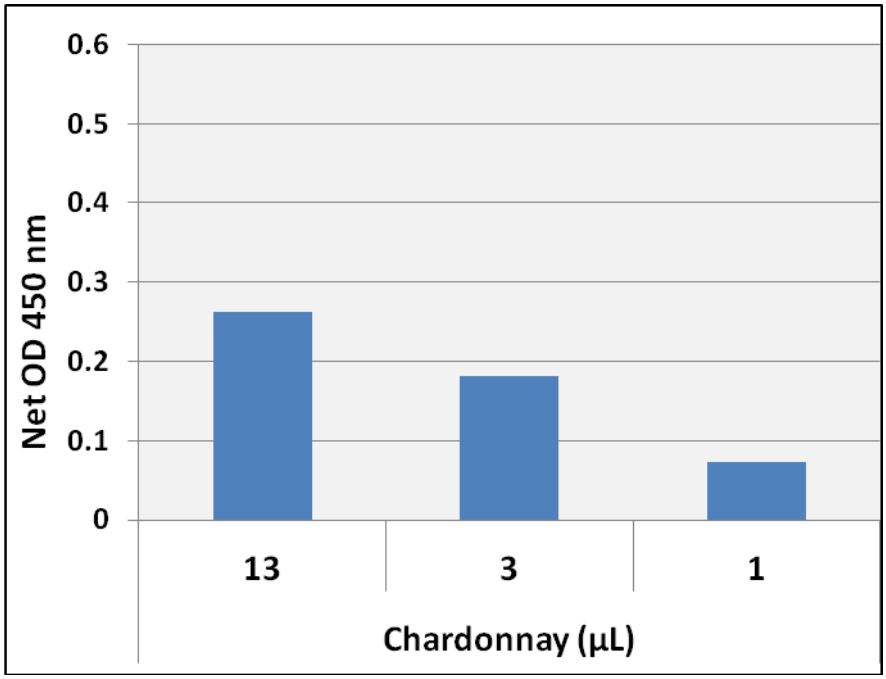

Figure 3. Detection of Malate in Chardonnay Wine. La Crema Chardonnay Sonoma Coast 2015 Wine was assayed according to the kit protocol.

Calculation of Results.

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without MDH (-MDH) from the sample well values containing enzyme (+MDH) to obtain the difference. The absorbance difference is due to the enzyme MDH activity:

$$\Delta A = A_{(+MDH)} - A_{(-MDH)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of malate present in the sample. Only use values within the range of the standard curve.

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.

Recent Product Citation

Tan, L. et al. (2022). Effects of Malate Ringer's solution on myocardial injury in sepsis and enforcement effects of TPP@PAMAM-MR. *J Transl Med*. **20**(1):591. doi: 10.1186/s12967-022-03811-y.

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.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com