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Product Information

Malate Assay Kit

Catalog Number **MAK067**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

L-Malate is a tricarboxylic acid cycle intermediate and a critical component of the malate-aspartate shuttle. The malate-aspartate shuttle is required for transporting reducing equivalents across the mitochondrial membrane for use in oxidative phosphorylation. In lower organisms, malate is converted to lactate during malolactic fermentation with the formation of CO_2. Malate is frequently used as an additive in the food and pharmaceutical industries, so quantitating malate is important in the manufacturing of beer, wine, cheese, and fruits.

The Malate Assay kit is an easy and sensitive assay to measure the malate level in a variety of samples. Malate concentration is determined by an enzymatic assay, which results in a colorimetric (450 nm) product, proportional to the malate present. Typical detection range for this kit is 5–25 nmoles of malate.

Components

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

Malate Assay Buffer Catalog Number MAK067A	20 mL
Malate Enzyme Mix Catalog Number MAK067B	1 vl
WST Substrate Mix Catalog Number MAK067C	1 vl
Malate Standard, 10 µmole Catalog Number MAK067D	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Malate Assay Buffer – Allow buffer to come to room temperature before use.

Malate Enzyme Mix – Reconstitute in 220 µL of water. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

WST Substrate Mix – Reconstitute in 1.05 mL of water. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Malate Standard – Reconstitute in 100 µL of water to generate 100 mM Standard Solution. Mix well by pipetting. Aliquot and store at –20 °C.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

Malate Standards for Colorimetric Detection

Dilute 10 µL of the 100 mM Malate Standard Solution with 990 µl of water to generate a 1 mM standard solution. Add 0 (blank), 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate, generating 0, 2, 4, 6, 8, and 10 nmole/well standards. Add Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Tissue samples (10–100 mg) should be rapidly homogenized with two volumes of ice cold PBS or other buffer (pH 6.5–8).

Solids should be processed by homogenizing 20 mg with 500 µL of water, with mild heating, for 30 minutes. Centrifuge at 10,000 × *g* for 10 minutes. After filtration, the soluble fraction can be assayed directly with appropriate dilution.

Most food or beverage samples can be assayed directly.

Samples may be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction. This step may be necessary if enzymes in the samples interfere with the assay. If protein, fat, or solids/particulates are present, samples should be filtered through a 10 kDa MWCO spin filter.

Add 1–50 µL samples into duplicate wells of a 96 well plate. Bring samples to final volume of 50 µL/well with Malate Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).
   Note: Reducing agents such as NADH or NADPH from cell or tissue extracts may generate background for the malate assay. To remove the effect of NADH or NADPH background, a blank sample may be set up by omitting the Malate Enzyme Mix. The blank sample readings can then be subtracted from the sample readings.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
Malate Assay Buffer	38 µL	40 µL
Malate Enzyme Mix	2 µL	–
WST Substrate Mix	10 µL	10 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate at 37 °C for 30 minutes, protected from light.

3. Measure the absorbance at 450 nm (A_{450}). The color is stable for up to 4 hours.

Results

Calculations

The background is the value obtained for the 0 (blank) malate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate malate standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample readings from the sample readings. The amount of malate present in the samples may be determined from the standard curve.

Concentration of Malate

$$C = S_a/S_v$$

C = Concentration of malate in sample
S_a = Amount of malate in unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells.

Malic acid molecular weight: 134.09 g/mole

Sample Calculation

Malate amount (S_a) = 22.07 nmole (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of malate in sample

$$22.07 \text{ nmole}/50\ \mu\text{L} = 0.44 \text{ nmole}/\mu\text{L}$$

$$0.44 \text{ nmole}/\mu\text{L} \times 134.09 \text{ ng/nmole} = 59.0 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Assay Buffer Cold	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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