Malate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1187

(Version 1.2A)

Detection and Quantification of MalateContentin Food, Juice, Beverage, Other agricultural productsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

L-malate, or L-Malic acid, 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.

MalateColorimetric Microplate Assay Kit is designed to directly measure malate content in a variety of samples. It isbased on malate dehydrogenasecatalyzed oxidation of malate in which the formed NADH reduces aformazan reagent. The intensity of the product color, measured at450 nm is proportional to the malate concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	10 mlx 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use.Aliquot & store at -80 °C.Use within 1 month.

Dye Reagent A: Add 9 mldistilled water to dissolve before use, mix. Store at 4 °C. Use within 1 month.

Standard: Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 50 mmol/L of standardstock solution, store at -20 °C for 1 monthafter reconstitution.Then dilute to10mmol/Lstandard top solutionby adding 100 µl stock solution into 400µl distilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

V. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mldistilled water, transfer it into microcentrifuge tube, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2.For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	80 µl	80 µl	80µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Dye Reagent A	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, keep in dark for 30 minutes at room temperature, record absorbance measured at 450 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-Intercept}{Slope}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.2According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

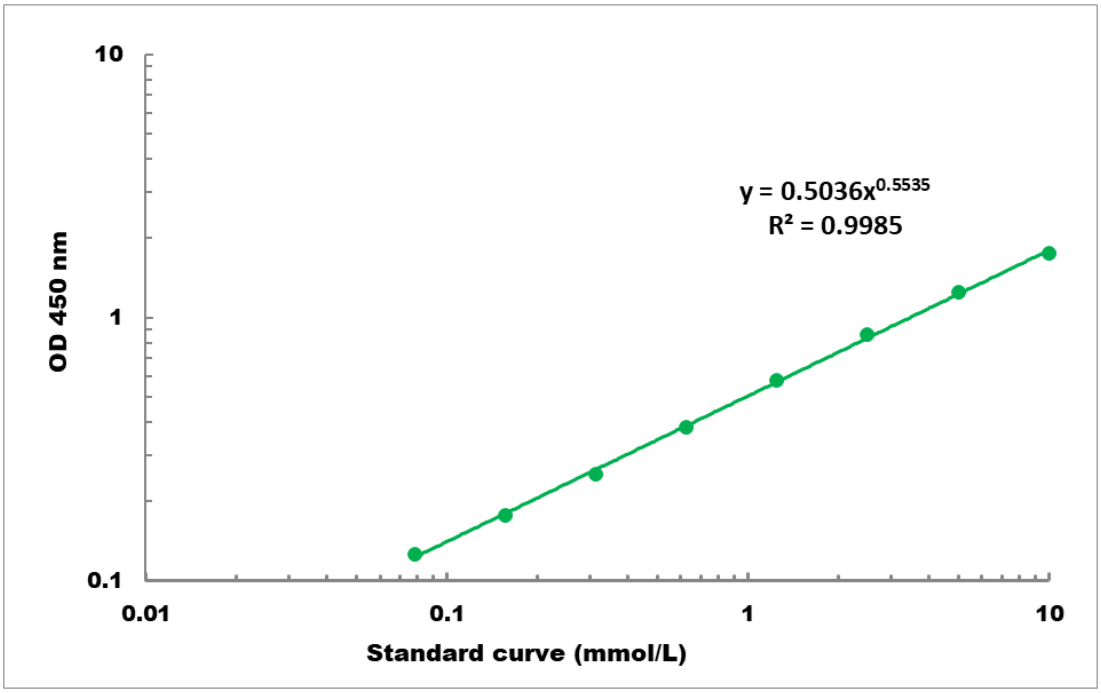
V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1mmol/L - 10mmol/L