CheKine™ Micro Malic Acid Content Assay Kit

Cat #: KTB1028 Size: 48 T/48 S 96 T/96 S

	Micro Malic Acid (Activity) Assay Kit		
REF	**Cat #:** KTB1028	LOT	**Lot #:** Refer to product label
	Detection range: 15.625-1000 nmol/mL		**Sensitivity:** 15.625 nmol/mL
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Malic acid is a natural organic acid widely present in plants, animals, and microorganisms, and is particularly abundant in unripe fruits. As a key intermediate metabolite in the tricarboxylic acid (TCA) cycle, malic acid plays important roles in cellular energy metabolism, maintaining redox balance, and regulating intracellular pH. Furthermore, malic acid is commonly used in the food industry as an acidity regulator and flavor enhancer, and also has wide applications in the pharmaceutical and cosmetic fields.

The principle is: Under the catalysis of malate dehydrogenase (MDH), malic acid undergoes a specific redox reaction with oxidized nicotinamide adenine dinucleotide (NAD^+), producing oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH). Subsequently, the generated NADH, mediated by the electron coupling reagent 1-methoxy-5-methylphenazinium methyl sulfate (1-mPMS), reduces the water-soluble tetrazolium salt WST-8 to an orange-yellow formazan product. This product exhibits a maximum absorption peak at 450 nm, and its formation is directly proportional to the concentration of malic acid in the sample .

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	60 mL	60×2 mL	4℃
Extraction Buffer Ⅱ	10 mL	20 mL	4℃
Reagent Ⅰ	15 mL	30 mL	4℃
Reagent Ⅱ	1.25 mL	2.5 mL	-20℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×2 vial	-20℃, protected from light
Reagent Ⅳ	300 μL	600 μL	-20℃, protected from light
100 mM Standard	1 mL	1 mL	-20℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm

- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, ice maker, centrifuge, Ultrasonic cell disruptor
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Extraction Buffer Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light. The remaining reagent can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.Keep on ice during the experiment.

Working Reagent Ⅲ: Prepared before use.Take one vial add 1.25 mL of Reagent Ⅰ to fully dissolve. The prepared reagent can be stored at -20℃, protected from light for 1 month.Keep on ice during the experiment.

Reagent Ⅳ: Ready to use as supplied; Keep on ice and protect from light during the assay. Store unused Reagent at -20℃, protected from light.

100 mM Standard: Ready to use as supplied; The remaining reagent can be stored at -20℃ after aliquoting to avoid repeated freezing and thawing; shake to mix before use.

Working Solution: For each assay well/blank well/standard well, prepare 90 μL of Assay Working Solution by mixing 9 μL Reagent Ⅱ, 9 μL Working Reagent Ⅲ, 2 μL Reagent Ⅳ, and 70 μL Reagent Ⅰ. Keep the prepared working solution on ice, protect from light as much as possible, and mix well before use. The working solution should be freshly prepared. For each control well, prepare 90 μL of control working solution by mixing 9 μL Reagent Ⅱ, 9 μL Working Reagent Ⅲ, and 72 μL Reagent Ⅰ. The control working solution should also be freshly prepared.

Standard preparation:

10 mM Standard: Prepare 10 mM Standard by mixing 10 μL of 100 mM Standard with 90 μL of Reagent Ⅰ, mix well to dilute. Using the 10 mM Standard, prepare standard curve dilution as described in the table:

Num.	Standard Volume	Reagent Ⅰ (μL)	Concentration (nmol/mL)
Std.1	50 μL 10 mM Standard	450	1000
Std.2	200 μL of Std.1 (1000 nmol/mL)	200	500
Std.3	200 μL of Std.2 (500 nmol/mL)	200	250
Std.4	200 μL of Std.3 (250 nmol/mL)	200	125
Std.5	200 μL of Std.4 (125 nmol/mL)	200	62.5
Std.6	200 μL of Std.5 (62.5 nmol/mL)	200	31.25
Std.7	200 μL of Std.6 (31.25 nmol/mL)	200	15.625
Blank	0	200	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: It is recommended to use fresh samples and extract them as soon as possible. If extraction cannot be performed on the same day, store intact cells or aliquoted tissue pieces at -80℃ and use within one month. When ready to perform the experiment, thaw the samples on ice after removing them from -80℃. However, please note that this may affect sample stability, and the experimental results may be lower than expected.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer Ⅰ and homogenize or mortar on ice. Centrifuge at 12,000 g for

10 min at 4℃. Take 800 μL supernatant, add 150 μL Extraction Buffer II and mix well slowly. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.

2. Bacteria or cells: Collect 5×10^6 bacteria or cells into the centrifuge tube, wash bacteria or cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer I to ultrasonically disrupt the bacteria or cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 10 min at 4℃. Take 800 μL supernatant, add 150 μL Extraction Buffer II and mix well slowly. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.

3. Plasma, Serum or other Liquid samples: Take 100 μL liquid sample, add 1 mL Extraction Buffer I and mix well. Centrifuge at 12,000 g for 10 min at 4℃. Take 800 μL supernatant, add 150 μL Extraction Buffer II and mix well slowly. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample. Because Extraction Buffer I and Extraction Buffer II can cause protein degeneration, the sample needs to be extracted with deionized water according to the steps separately.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Test Well (μL)	Control Well (μL)	Blank Well (μL)	Standard Well (μL)
Sample	10	10	0	0
Standard	0	0	0	10
Reagent I	0	0	10	0
Assay Working Solution	90	0	90	90
Control Working Solution	0	90	0	0

3. Mix well, Incubate for exactly 30 min at 37℃ in the dark. The absorbance value is measured at 450 nm. The Test Well is marked as A_{Test}, the Control Well is recorded as $A_{Control}$, the Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, and . Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note:The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.015, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1,000 nmol/mL of $\Delta A_{Standard}$, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculation of the Malic Acid content

Bring the ΔA_{Test} of the sample into the equation to get the y value (nmol/mL)

(1) Calculated by protein concentration

Malic Acid (nmol/mg prot)=$V_{Sample}\times y\div(V_{Sample}\times Cpr)\times n$**=$y\div Cpr\times n$**

(2) Calculated by fresh weight of samples

Malic Acid (nmol/g fresh weight)=$(V_{Supernatant}+V_{Extr\ II})\times y\div(w\times V_{Supernatant}\div V_{Extr\ I})\times F$**=$1.1875y\div w\times n$**

(3) Calculated by number of bacteria or cells

Malic Acid (nmol/10^6)=($V_{Supernatant}$+$V_{Extr\ II}$)×y÷(n×$V_{Supernatant}$÷$V_{Extr\ I}$)×n**=1.1875y÷N×n**

(4) Calculated by volume of liquid samples

Malic Acid (nmol/mL)=($V_{Supernatant}$+$V_{Extr\ II}$)×y÷[V_{Liquid}×$V_{Supernatant}$÷(V_{Liquid}+$V_{Extr\ I}$)]×n**=13.0625y×n**

V_{Sample}: Added the sample volume, 0.05 mL; $V_{Supernatant}$: Volume of supernatant during extraction, 0.8 mL; $V_{Extr\ I}$: Added Extraction Buffer Ⅰ volume, 1 mL; $V_{Extr\ II}$: Added Extraction Buffer Ⅱ volume, 0.15 mL; V_{Liquid}: Volume of liquid sample, 0.1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; N: Number of bacteria or cells, calculated in units of one million; n: Sample dilution multiple.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

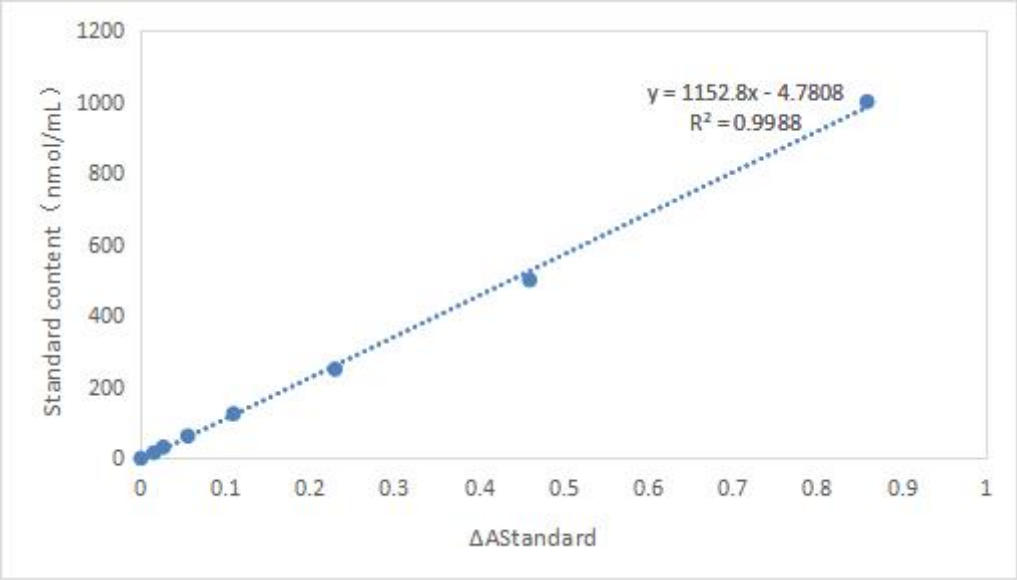

Figure 1. Standard Curve of Malic Acid.

Recommended Products

Catalog No.	Product Name
KTB1241	CheKine™ Micro α-Ketoglutaric Acid(α-KG)Content Assay Kit
KTB1230	CheKine™ Micro Succinate Dehydrogenase（SDH) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit
KTB1023	CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.