（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K905-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader (445-465 nm)

Detection range：0.003-0.5 mmol/L

Elabscience® Malic Acid Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 4

Materials prepared by users 5

Reagent preparation 5

Sample preparation 7

Operating steps 8

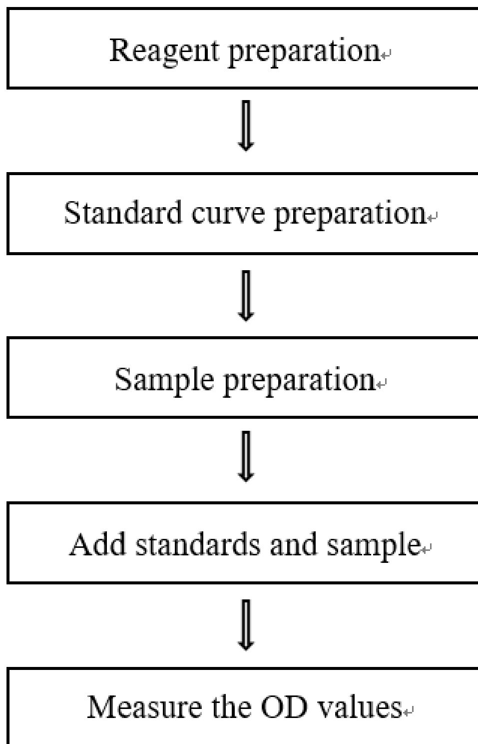
Calculation 9

Appendix I Performance Characteristics 10

Appendix Π Example Analysis 12

Statement 13

Assay summary



Intended use

This kit can measure malic acid content in serum (plasma), tissue and cell samples.

Detection principle

L-malic acid, also known as 2-hydroxysuccinic acid, is widely present in living organisms. It has two stereoisomers and is one of the members of the tricarboxylic acid cycle, an important metabolic cycle in living organisms. The detection principle of this kit is that the enzyme catalyzes malic acid to produce oxaloacetic acid. At the same time, under the action of electronic coupler, the chromogenic agent is reduced to produce orange yellow product, which has the maximum absorption peak at about 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20 ℃, 12 months
Reagent 2	Enzymatic Reagent	0.3 mL × 1 vial	-20 ℃, 12 months, shading light
Reagent 3	Chromogenic Agent	2.4 mL × 1 vial	-20 ℃, 12 months, shading light
Reagent 4	Substrate	Powder × 2 vials	-20 ℃, 12 months, shading light
Reagent 5	0.5 mmol/L Standard Solution	5 mL × 1 vial	-20 ℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (445-465 nm, optimum wavelength: 450 nm), Incubator(37°C), 10 kD Ultrafiltration tube

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of enzymatic working solution:

Before testing, please prepare sufficient enzymatic working solution according to the test wells. For example, prepare 40 μL of enzymatic working solution (mix well 2 μL of enzymatic reagent and 38 μL of buffer solution). The enzymatic working solution should be prepared on spot. Keep enzymatic working solution on ice for use.

③ Preparation of substrate working solution:

Dissolve one vial of substrate with 1.5 mL of double distilled water, mix well to dissolve. Store at -20 ℃ for 7 days.

④ Preparation of working solution:

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 30 μL of working solution (mix well 20 μL of enzymatic working solution, 20 μL of substrate working solution and 80 μL of buffer solution). The working solution should be prepared on spot. Keep working solution on ice protected from light for use.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.3, 0.35, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.35**	**0.4**	**0.5**
0.5 mmol/L standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: Take 100-500 μL serum(plasma) sample, centrifuge the supernatant with a 10 kD ultrafiltration tube at 12000×g for 15 min, take the filtrate from the outer tube for use.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000×g for 10 min at 4 ℃ to remove insoluble material. Take 100-500 μL supernatant, centrifuge the supernatant with a 10 KD ultrafiltration tube at 12000 g×g for 15min at 4°C, take the filtrate from the outer tube for use.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 2×10^6 cells in 400 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4 ℃.

④ Centrifuge at 10000×g for 10 min at 4 ℃ to remove insoluble material. Take 200 μL supernatant, centrifuge the supernatant with a 10 KD ultrafiltration tube at 12000×g for 15min at 4 ℃, take the filtrate from the outer tube for use.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat liver tissue homogenate	3-5
10% Mouse liver tissue homogenate	3-5
10% Lemon tissue homogenate	8-12
10% Tangerine tissue homogenate	5-10
10% Potato tissue homogenate	5-12
Rat serum	1
Mouse plasma	1
3.35×10^6 CHO cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Take 20 μL of standard solution with different concentrations to the standard well.
Sample well: Take 20 μL of sample to the sample well.
Control well: Take 20 μL of sample to the control well.

② Add 120 μL of working solution to the standard well and sample well. Add 120 μL of buffer solution to the control well.

③ Add 20 μL of chromogenic agent to each well.

④ Mix fully with microplate reader for 5 s and incubate at 37°C for 30 min with shading light. Measure the OD value of each well at 450 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

$$\text{malic acid content (mmol/L)} = (\Delta A_{450} - b) \div a \times f$$

2. Tissue sample:

$$\text{malic acid content (mmol/kg wet weight)} = (\Delta A_{450} - b) \div a \div (m \div V) \times f$$

3. Cell sample:

$$\text{malic acid content (mmol/10^6)} = (\Delta A_{450} - b) \div a \div (n \div V) \times f$$

[Note]

ΔA_{450}: $OD_{Sample} - OD_{Control.}$

f: Dilution factor of sample before test.

m: The weight of the sample, g.

V: The volume of homogenate, mL.

n: The number of cell sample/10^6

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.06	0.10	0.30
%CV	3.6	3.4	2.6

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.06	0.10	0.30
%CV	4.6	5.9	5.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.13	0.27	0.38
Observed Conc. (mmol/L)	0.1	0.3	0.4
Recovery rate (%)	101	96	97

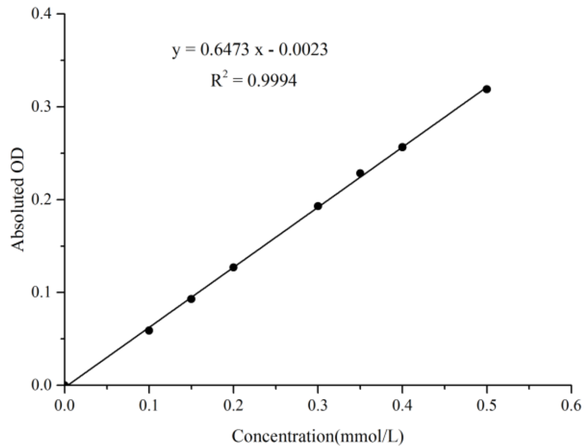
Sensitivity

The analytical sensitivity of the assay is 0.003 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	0	0.1	0.15	0.2	0.3	0.35	0.4	0.5
Average OD	0.064	0.130	0.157	0.191	0.257	0.292	0.320	0.383
Absoluted OD	0	0.059	0.093	0.127	0.193	0.229	0.257	0.319



Appendix Π Example Analysis

Example analysis:

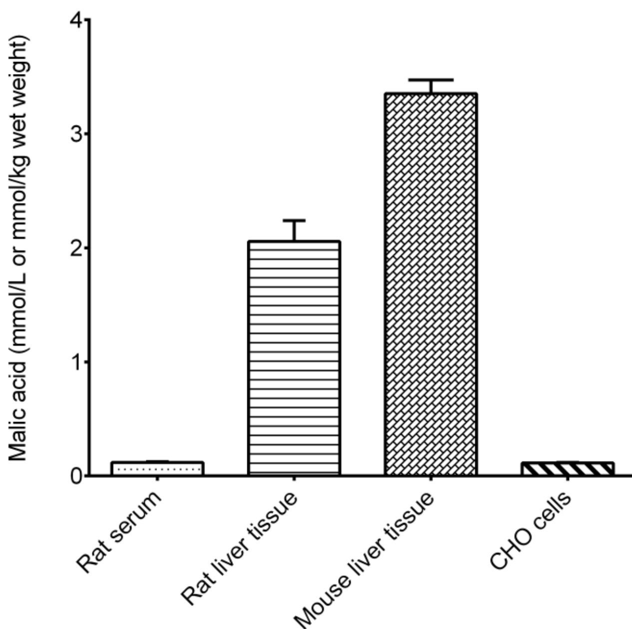
For 10% mouse liver tissue homogenate, dilute for 4 times, take 20 μL and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.6473x - 0.0023, the average OD value of the sample is 0.120, the average OD value of the control is 0.081, $\Delta A_{450} = A_{sample} - A_{control} = 0.120 - 0.081 = 0.039$, and the calculation result is:

malic acid content (mmol/kg wet weight)

$= (0.039 + 0.0023 \div 0.6473 \div (0.1 \div 0.9) \times 4 = 2.297$ mmol/kg wet weight

Detect rat serum, 10% rat liver tissue homogenate (dilute for 4 times), 10% mouse liver tissue homogenate (dilute for 4 times) and 3.35×106 CHO cells according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.