TECHNICAL MANUAL

Malate Acid Colorimetric Assay Kit

- **SKU CODE:** MAES0281
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure absorbance

2. Intended use

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

3. 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 an orange-yellow product, which has the maximum absorption peak at about 450 nm.

4. Kit components & storage

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

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzymatic working solution:** Before testing, 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°C for 7 days.
4. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the test wells. For example, prepare 120 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.

7. Standard curve preparation

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

Item	1	2	3	4	5	6	7	8
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

8. Sample preparation

Sample preparation:

Serum and plasma: Take 100-500 μL serum (plasma) sample, centrifuge the supernatant with a 10 kD ultrafiltration tube at 12,000×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°C.

4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Take 100-500 μL supernatant, centrifuge the supernatant with a 10 kD ultrafiltration tube at 12,000×g for 15 min 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 an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Take 200 μL supernatant, centrifuge the supernatant with a 10 kD ultrafiltration tube at 12,000×g for 15 min at 4°C, take the filtrate from the outer tube for use.

Dilution of sample:

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

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 perform a pretest to confirm the dilution factor.

9. 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.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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:

malic acid content (mmol/L) = (ΔA_450 - b) ÷ a × f

2. Tissue sample:

malic acid content (mmol/kg wet weight) = (ΔA_450 - b) ÷ a ÷ (m ÷ V) × f

3. Cell sample:

malic acid content (mmol/10^6) = (ΔA_450 - b) ÷ a ÷ (n ÷ V) × 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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision Data

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.

Inter-assay Precision Data

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

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration assayed 6 times in parallel to get an average recovery rate of 98%.

Recovery Data

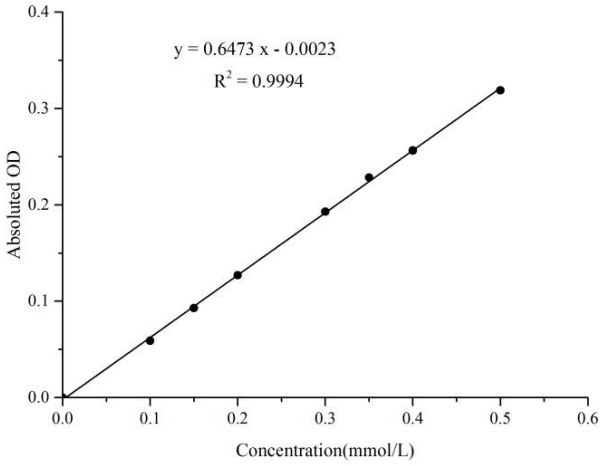
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.13	0.27	0.38
Observed Conc. (mmol/L)	0.1	0.3	0.4
Recovery rate (%)	101	96	97

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.

Standard curve data

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), the standard curve and data are provided as below for reference only:



Standard curve reference data

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

12. Appendix II Example Analysis

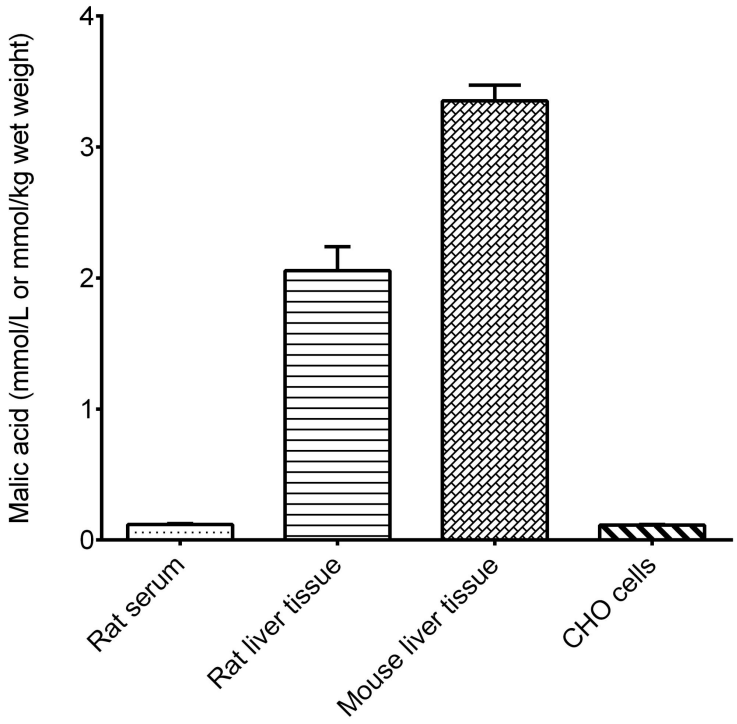
Example analysis:

For 10% mouse liver tissue homogenate, dilute 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) ÷ 0.6473 ÷ (0.1 ÷ 0.9) × 4 = 2.297 mmol/kg wet weight

Detect rat serum, 10% rat liver tissue homogenate (dilute 4 times), 10% mouse liver tissue homogenate (dilute 4 times) and 3.35×10^6 CHO cells according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purposes.

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 manual.

6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Assay Genie will guarantee the quality of the kits only, and will 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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.