TECHNICAL MANUAL

Oxaloacetate Colorimetric Assay Kit

- **SKU CODE:** MAES0273
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can measure oxaloacetate content in animal tissue samples.

2. Detection principle

Oxaloacetic acid (OAA), one of the intermediates in the tricarboxylic acid cycle, is an important substance in carbon and nitrogen metabolism.

Oxaloacetate reacts with substrate acetyl-coA under the action of enzyme. The substance produced can react with DTNB, which has the maximum absorption peak at 412 nm. The content of oxaloacetate can be determined by measuring the absorbance value.

3. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL x 1 vial	20 mL x 1 vial	-20 °C, 12 months
Reagent 2	Substrate	1 mL x 1 vial	2 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 3	Enzyme Reagent	Liquid x 1 vial	Liquid x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Chromogenic Agent	1.2 mL x 1 vial	2.4 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 5	Standard	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

4. Materials prepared by users

Instruments:

Microplate reader (402-422 nm, optimum wavelength: 412 nm), Incubator, Ultrafiltration tube (50kD)

Reagents:

Double distilled water

5. Reagent preparation

1. Equilibrate other reagents to room temperature before use.
2. **Preparation of substrate working solution::** For each well, prepare 140 µL of substrate working solution (mix well 126 µL of buffer solution and 14 µL of substrate). Keep substrate working solution on ice protected from light during use. Aliquoted storage at -20 °C protected from light, and the prepared solution should be used up within 7 days.
3. **Preparation of enzyme working solution::** Dissolve one vial of enzyme reagent with 1170 µL of double distilled water, mix well to dissolve. Keep enzyme working solution on ice protected from light during use. The prepared solution should be used up within 2 weeks.
4. **Preparation of 50 mmol/L standard::** Dissolve one vial of standard with 1 mL of double distilled water, mix well to dissolve. Keep 50 mmol/L standard on ice protected from light during use. Store at -20 °C for 7 days protected from light.
5. **Preparation of 1 mmol/L standard::** Before testing, please prepare sufficient 1 mmol/L standard according to the test wells. For example, prepare 800 µL of 1 mmol/L standard (mix well 16 µL of 50 mmol/L standard and 784 µL of double distilled water). The 1 mmol/L standard should be prepared on spot.
6. **The preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.5, 0.6, 0.8, 1.0, mmol/L.

6. Standard curve preparation

Item	Concentration (mmol/L)	1 mmol/L standard (µL)	Double distilled water (µL)
①	0	0	200
②	0.1	20	180
③	0.2	40	160
④	0.3	60	140
⑤	0.5	100	100

Item	Concentration (mmol/L)	1 mmol/L standard (μL)	Double distilled water (μL)
⑥	0.6	120	80
⑦	0.8	160	40
⑧	1.0	200	0

7. Sample preparation

Sample preparation

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 12,000 x g for 15 min at 4 °C to remove insoluble material. Collect supernatant for centrifugation with a 50 kD ultrafiltration tube at 10,000 x g for 15 min, and preserve the filtrate on ice for detection.

Dilution of sample:

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

10% Rat liver tissue homogenate: 1

10% Rat brain tissue homogenate: 1

10% Rat lung tissue homogenate: 1

10% Mouse liver tissue homogenate: 1

10% Mouse kidney tissue homogenate: 1

10% Mouse leg muscle tissue homogenate: 1

10% Pig heart tissue homogenate: 1

10% Human urine tissue homogenate: 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.

8. The key points of the assay

1. All reagents should be stored with shading light strictly.
2. Mix chromogenic agent fully before use and preserve it on the ice box away from light during use.
3. Don't dissolve buffer solution with heating and avoid repeated freeze-thaw.

9. Operating steps

1. Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells. Sample well: Add 20 μL of sample to the corresponding wells.
2. Add 140 μL of substrate working solution to each well.
3. Add 20 μL of enzyme working solution to each well.
4. Add 20 μL of chromogenic agent to each well.
5. Mix fully with microplate reader for 5 s and incubate at room temperature protected from light for 3 min. Measure the OD value of each well at 412 nm with microplate reader.

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:

OAA (mmol/kg wet weight) = (ΔA - b) ÷ a ÷ (m ÷ V) × f

[Note]

ΔA: $OD_{sample} - OD_{blank}$.

m: The weight of the sample, g.

V: The volume of homogenate, mL

f: Dilution factor of sample before test.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.06	0.24	0.75
%CV	2.5	2.2	1.6

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.06	0.24	0.75
%CV	2.4	2.2	2.3

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 101%.

Recovery

Standard	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Standard 1	0.15	0.2	101
Standard 2	0.45	0.4	99

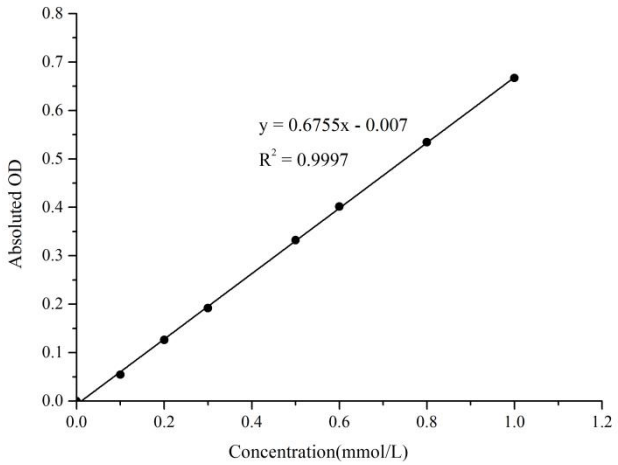
Standard	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Standard 3	0.7	0.7	103

Sensitivity

The analytical sensitivity of the assay is 0.017 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

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:



Standard curve data

Concentration (mmol/L)	Average OD	Absolute OD
0	0.278	0
0.1	0.333	0.055
0.2	0.405	0.127
0.3	0.470	0.192
0.5	0.610	0.332

Concentration (mmol/L)	Average OD	Absolute OD
0.6	0.680	0.402
0.8	0.813	0.535
1.0	0.945	0.667

12. Appendix II Example Analysis

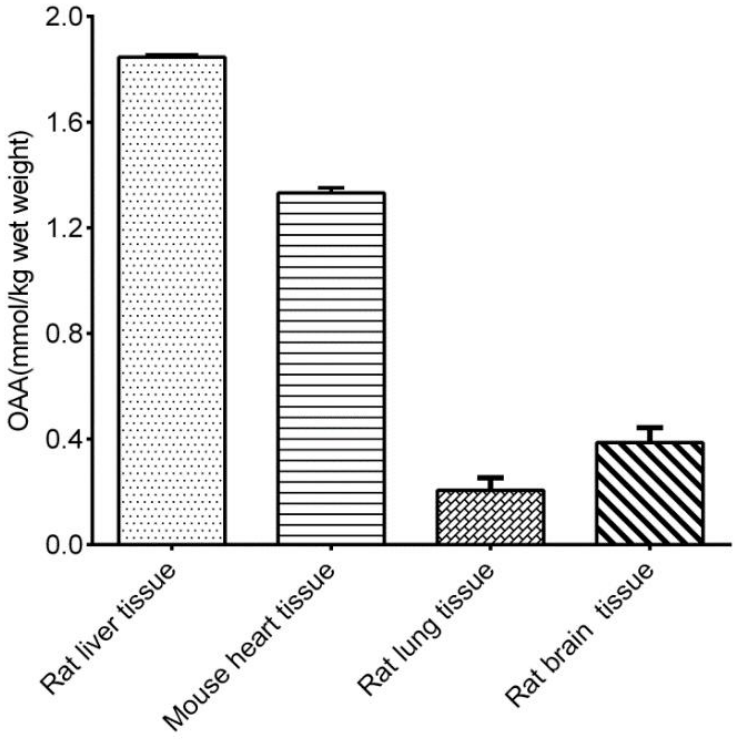
Example analysis:

For 10% Rat liver tissue homogenate, take 20 µL of sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.6755 x - 0.007, the average OD value of the control is 0.278, the average OD value of the sample is 0.410, and the calculation result is:

OAA (mmol/kg wet weight) = (0.410 – 0.278+0.007) ÷ 0.6755 ÷ (0.1 ÷ 0.9) = 1.85 mmol/kg wet weight

Detect 10% Rat liver tissue homogenate (take 20 µL of the supernatant), 10% Mouse heart tissue homogenate (take 20 µL of the supernatant), 10% Rat lung tissue homogenate (take 20 µL of the supernatant), 10% Rat brain tissue homogenate (take 20 µL of the supernatant) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Please follow the instructions strictly during the experiments.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.