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

Catalog No：E-BC-K901-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (402-422 nm)

Detection range：0.017-1 mmol/L

Elabscience®Oxaloacetate(OAA)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.

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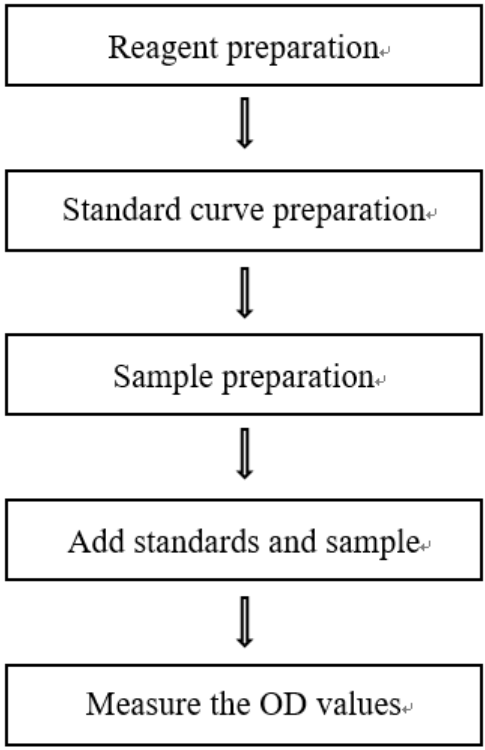
Assay summary



Intended use

This kit can measure oxaloacetate content in animal tissue samples.

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.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL ×1 vial	20 mL ×1 vial	-20℃, 12 months
Reagent 2	Substrate	1 mL ×1 vial	2 mL ×1 vial	-20℃, 12 months, shading light
Reagent 3	Enzyme Reagent	Liquid ×1 vial	Liquid ×2 vials	-20℃, 12 months, shading light
Reagent 4	Chromogenic Agent	1.2 mL ×1 vial	2.4 mL ×1 vial	-20℃, 12 months, shading light
Reagent 5	Standard	Power ×1 vial	Power ×2 vials	-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 (402-422 nm, optimum wavelength: 412 nm), Incubator, Ultrafiltration tube (50kD)

Reagents:

Double distilled water

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② Preparation of substrate working solution:

For each well, prepare 140 uL 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 ℃ 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 ℃ 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.5**	**0.6**	**0.8**	**1.0**
1 mmol/L standard (μL)	0	20	40	60	100	120	160	200
Double distilled water (μL)	200	180	160	140	100	80	40	0

Sample preparation

① 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 ℃.

④ Centrifuge at 12000 ×g for 15 min at 4 ℃ to remove insoluble material. Collect supernatant for centrifugation with a 50 kD ultrafiltration tube at 10000 ×g for 15 min, and preserve the filtrate on ice for detection.

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	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.

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.

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.

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:

$$\frac{\text{OAA}}{\text{(mmol/kg wet weight)}} = (\Delta A - b) \div a \div (m \div V) \times f$$

[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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat liver 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	0.24	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.

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L）	0.15	0.45	0.7
Observed Conc. (mmol/L）	0.2	0.4	0.7
Recovery rate (%)	101	99	103

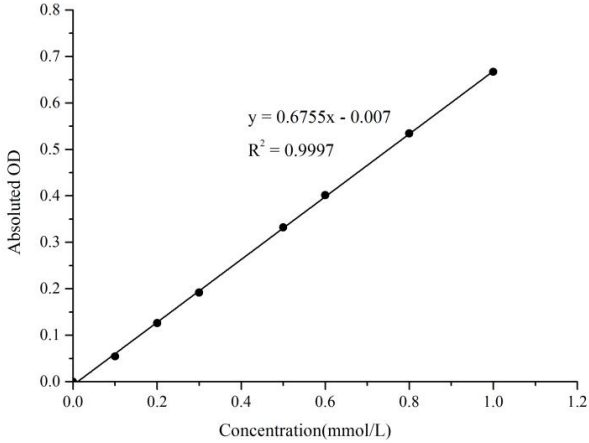
Sensitivity

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.

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**	**0.2**	**0.3**	**0.5**	**0.6**	**0.8**	**1.0**
Average OD	0.278	0.333	0.405	0.470	0.610	0.680	0.813	0.945
Absoluted OD	0	0.055	0.127	0.192	0.332	0.402	0.535	0.667



Appendix II Example Analysis

Example analysis:

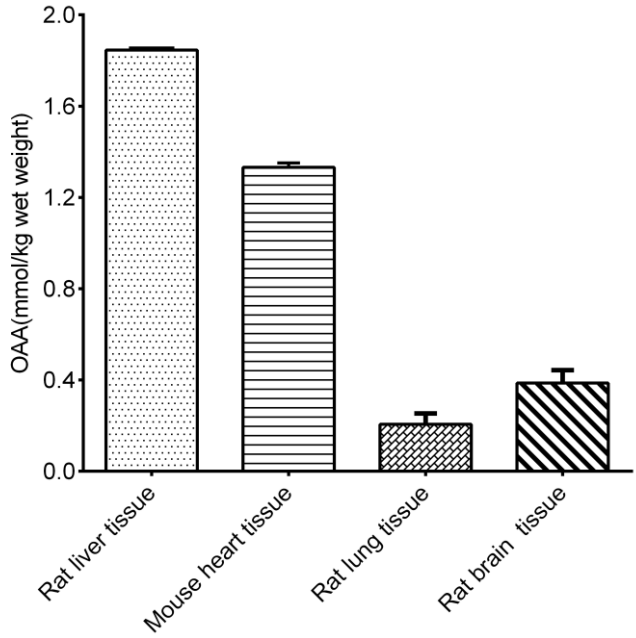
For 10% Rat liver tissue homogenate, take 20 μL of sample and carry the assay according to the operation table. The results are as follows:

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:



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.