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

Catalog No：E-BC-K652-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader（330-350 nm）

Detection range：150-500 nmol/mL

Elabscience®Acetyl-CoA 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 6

The key points of the assay 7

Operating steps 7

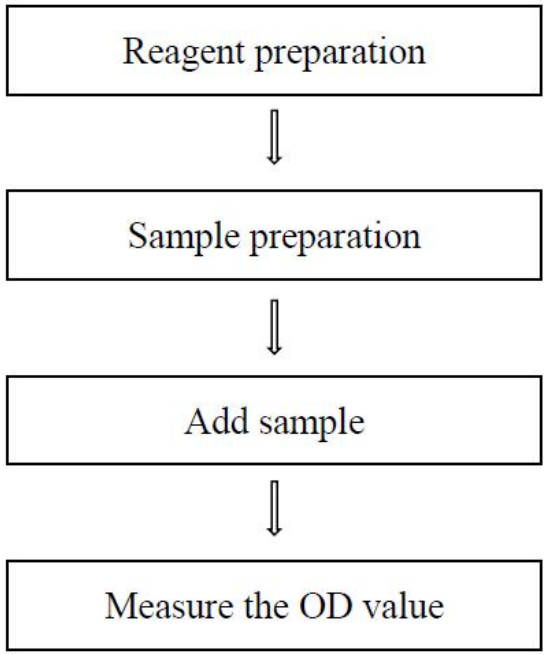
Calculation 8

Appendix I Performance Characteristics 9

Appendix II Example Analysis 10

Statement 11

Assay summary



Intended use

This kit can measure acetyl-coA content in animal tissue sample.

Detection principle

Malate dehydrogenase can catalyze malate and NAD^+ to produce NADH and oxoacetate, which reacts with acetyl-coa to form coenzyme A and citrate under the action of citrate synthetase. The content of acetyl-coA can be calculated by measuring the change of absorbance value at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	60 mL × 2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	20 mL × 1 vial	40 mL × 1 vial	-20℃, 12 months
Reagent 3	Enzyme Reagent A	Liquid × 1 vial	Liquid × 2 vials	-20℃, 12 months, shading light
Reagent 4	Enzyme Reagent B	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 5	Substrate	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 6	500 nmol/mL Standard	0.16 mL×1 vial	0.32 mL×1 vial	-20℃, 12 months, shading light
	UV 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 (330-350 nm, optimum wavelength: 340 nm)

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of enzyme reagent A working solution：
Dissolve one vial of enzyme reagent A with 500 μL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 3 days protected from light, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of enzyme reagent B working solution：
Dissolve one vial of enzyme reagent B with 500 μL of double distilled water, mix well to dissolve. Storage at 2-8°C for 3 days protected from light.

④ The preparation of substrate working solution：
Dissolve one vial of substrate with 500 μL of buffer solution, mix well to dissolve. Storage at 2-8°C for 3 days protected from light.

⑤ The preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 436 μL of reaction working solution (mix well 400 μL of buffer solution, 14 μL of enzyme reagent A working solution, 10 μL of enzyme reagent B working solution and 12 μL of substrate working solution, mix well. The reaction working solution should be prepared on spot. Keep reaction working solution at room temperature protected from light for 10 min before use.

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 extracting solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it 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 lung tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse kidney tissue homogenate	1

Note: The diluent is extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The reaction rate of the detection process is fast, so it is necessary to start to measure the A_1 immediately after adding the reaction working solution, otherwise the determination result is low..

② Preserve reaction working solution at room temperature with shading light for 10 min before use.

③ It's better to measure no more than 5 samples at same time.

Operating steps

① Standard well: Add 30 μL of standard solution to the corresponding wells.
Blank well: Add 30 μL of extracting solution to the corresponding wells.
Sample well: Add 30 μL of sample to the corresponding wells.

② Add 230 μL of reaction working solution to each well.

③ Mix fully with microplate reader for 3 s. Measure the OD value of each well at 0 and 1 min respectively at 340 nm with microplate reader, respectively recorded as A_1, A_2, $\Delta A=A_2-A_1$.

Calculation

The sample:

Tissue sample:

$$\text{Acetyl-CoA content (nmol/g wet weight)} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times 500^{*} \times V \div m \times f$$

[Note]

ΔA_{Sample}: The change OD value of sample well.

$\Delta A_{Standard}$: The change OD value of standard well.

ΔA_{Blank}: The change OD value of blank well.

500*： The concentration of standard, 500 nmol/mL.

m: The weight of wet tissue (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 tissue 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 (nmol/mL)	225.00	348.00	405.00
%CV	4.4	4.0	3.6

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mL)	225.00	348.00	405.00
%CV	5.0	5.2	5.7

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 103%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (nmol/mL)	185	279	452
Observed Conc. (nmol/mL)	192.4	287.4	461.0
Recovery rate (%)	104	103	102

Sensitivity

The analytical sensitivity of the assay is 150 nmol/mL. 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.

Appendix II Example Analysis

Example analysis：

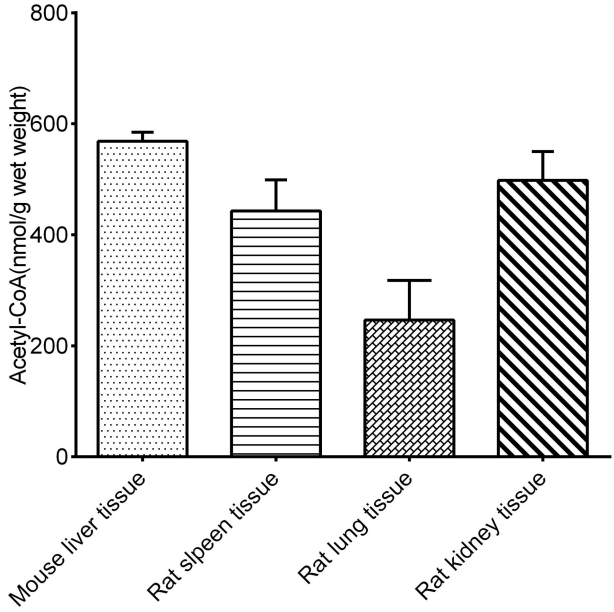
For mouse liver tissue, take 30 μL of 10% mouse liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

the OD value of the sample A1 is 0.271, the OD value of the sample A2 is 0.282, the OD value of the blank A1 is 0.07, the OD value of the blank A2 is 0.07, the OD value of the standard A1 is 0.194, the OD value of the standard A2 is 0.283, and the calculation result is:

$$\text{Acetyl-CoA (nmol/g wet weight)} = ((0.011 - 0) \times 500) \div 0.089 \times 0.9 \div 0.1$$

$$= 556.18 \text{ nmol/g wet weight}$$

Detect 10% mouse liver tissue homogenate, 10% rat spleen tissue homogenate, 10% rat lung tissue homogenate and 10% rat kidney tissue homogenate 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.