Technical Manual

Acetyl-CoA Colorimetric Assay Kit

- **Catalogue Code: MAES0238**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

150-500 nmol/L

Sensitivity:

150 nmol/L

Storage:

-20°C for 12 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 12 months.

Do not use components from different batches of kit.

2. Background:

Acetyl-CoA is a critical molecule in cellular metabolism, serving as a key substrate for the citric acid cycle and fatty acid synthesis. It consists of an acetyl group linked to coenzyme A and plays a vital role in energy production. Acetyl-CoA is formed from carbohydrates, fats, and proteins and is involved in the synthesis of various biomolecules.

3. Intended Use:

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

4. 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.

5. Kit Components & Storage:

Item	Specification	Storage
Extracting Solution	60 mL ×2 vials	-20°C, 12 months
Buffer Solution	40 mL ×1 vial	-20°C, 12 months
Enzyme Reagent A	Liquid ×2 vials	-20°C, 12 months, shading light
Enzyme Reagent B	Power ×2 vials	-20°C, 12 months, shading light
Substrate	Power ×2 vials	-20°C, 12 months, shading light
500 nmol/mL Standard	0.32 mL×1 vial	-20°C, 12 months, shading light
UV Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (330-350 nm, optimum wavelength: 340 nm)
- Double distilled water

6. Assay Notes:

1. The reaction rate of the detection process is fast, so it is necessary to start to measure the A1 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 minutes before use.
3. It's better to measure not more than 5 samples at a time.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.
2. 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. 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. 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. 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 the spot. Keep reaction working solution at room temperature protected from light for 10 min before use.

8. 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°C.
4. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

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 buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

9. Operation 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 A1, A2, ΔA=A2-A1.

10. Calculations:

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.

11. Performance Characteristics:

Detection Range	150-500 nmol/L
Sensitivity	150 nmol/L
Average recovery rate (%)	103
Average inter-assay CV (%)	5.3
Average intra-assay CV (%)	4.0

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:

$$\text{Acetyl-CoA (nmol/g wet weight)} = ((0.011 - 0) \times 500) \div 0.089 \times 0.9 \div 0.1$$
$$= 556.18 \text{ nmol/g wet weight}$$

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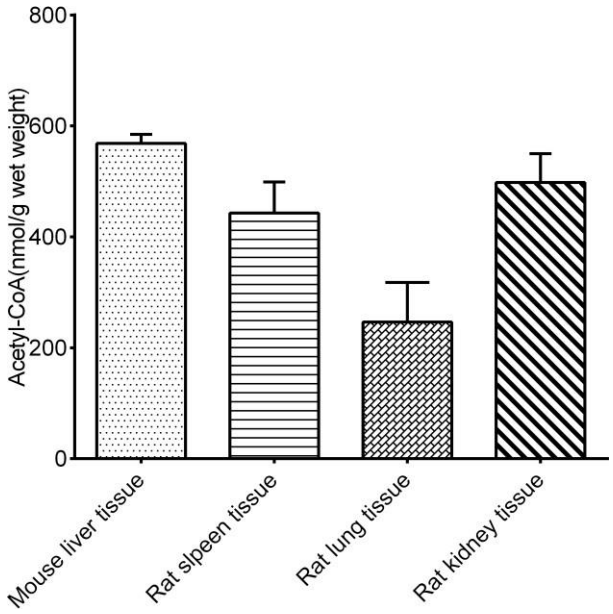
Detect 10% mouse liver tissue homogenate, 10% rat spleen tissue homogenate, 10% rat lung tissue homogenate and 10% rat kidney tissue homogenate according to the protocol, the result is as follows:



Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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