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

Catalog No：E-BC-F046

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.27-5.00 μmol/L

Elabscience® Acetyl Coenzyme A (Acetyl-CoA) Fluorometric 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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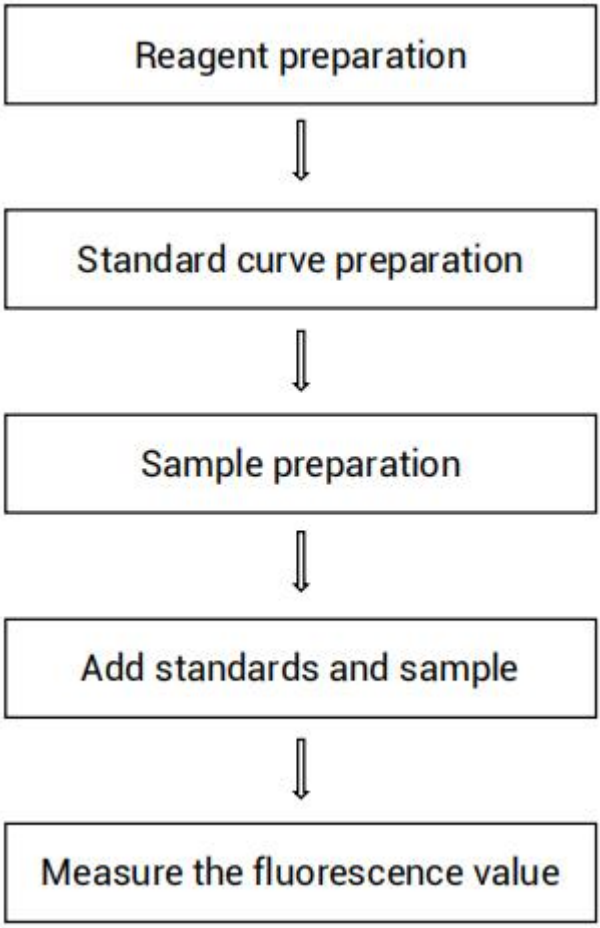
Assay summary



Intended use

This kit can be used to measure acetyl coenzyme A (Acetyl-CoA) content in serum (plasma), animal tissue and cell samples.

Detection principle

Acetyl-CoA is an essential cofactor and acyl carrier in the enzymatic acetyl transfer reaction. It can be formed by oxidative decarboxylation of pyruvate in mitochondria, oxidation of long-chain fatty acids, or oxidative degradation of certain amino acids. Acetyl-CoA is the starting compound of the citric acid cycle.

The detection principle of this kit is that Acetyl-CoA is converted into other substances under the condition of enzyme catalysis, and the resulting fluorescence is detected by fluorescence microplate reader at the maximum excitation wavelength of 535 nm and the maximum emission wavelength of 587 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	40 mL × 1 vial	40 mL × 2 vials	-20°C, 12 months shading light
Reagent 2	Buffer Solution	24 mL × 1 vial	48 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Cofactor	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months shading light
Reagent 4	Substrate	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months shading light
Reagent 5	Enzyme Solution	0.5 mL × 1 vial	1.0 mL × 1 vial	-20°C, 12 months shading light
Reagent 6	Enzyme Reagent	0.36 mL × 1 vial	0.72 mL × 1 vial	-20°C, 12 months shading light
Reagent 7	Fluorescence Probe	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months shading light
Reagent 8	500 μmol/L Standard	0.5 mL × 1 vial	0.5 mL × 1 vial	-20°C, 12 months shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm)

Consumptive material:

10kDa MWCO Spin Filter

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of co-factor working solution：
Dissolve one vial of co-factor with 8 mL of buffer solution, mix well to dissolve. Store at -20°C for 3 days.

③ The preparation of substrate working solution：
Dissolve one vial of substrate with 7.5 mL of co-factor working solution, mix well to dissolve. Store at -20°C for 3 days. (When opening the cover, be careful not to spill the co-factor and substrate).

④ The preparation of enzyme working solution：
For each well, prepare 70 μL of enzyme working solution (mix well 8 μL of enzyme solution, 6 μL of enzyme reagent and 56 μL of buffer solution). The enzyme working solution should be prepared on spot and used for same day.

⑤ The preparation of 5 μmol/L standard solution：
Before testing, please prepare sufficient 5 μmol/L standard solution. For example, prepare 1000 μL of 5 μmol/L standard solution (mix well 10 μL of 500 μmol/L standard and 990 μL of double distilled water). Keep it on ice during use. The remaining 500 μmol/L standard can be divided to avoid repeated freezing and thawing. The stability of 5 μmol/L standard decreased after dilution, and is used with 4 hours.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 5 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 3, 3.5, 4, 4.5, 5 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**1**	**2**	**3**	**3.5**	**4**	**4.5**	**5**
5 μmol/L Standard (μL)	0	40	80	120	140	160	180	200
Double distilled water (μL)	200	160	120	80	60	40	20	0

Sample preparation

① Sample preparation

Serum and plasma: Add 200-400 μL of serum(plasma) sample into 10kDa MWCO Spin Filter and centrifuge at 12000×g for 20 min. Collect the filtrate and preserve it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).
② Wash tissue in cold PBS (0.01 M, pH 7.4).
③ Homogenize 100 mg tissue in 900 μL extracting solution with a dounce homogenizer at 4°C.
④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.
⑤ Collect 200-400 μL supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 20 min at 4℃.
⑥ Take the filtered sample supernatant and preserve it on ice for detection.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
② Centrifuge at 400×g for 5 min at 4℃.
③ Homogenize 1×10^6 cells in 200 μL extracting solution with a dounce homogenizer at 4°C.
④ Centrifuge at 10000×g for 5 min at 4℃ to remove insoluble material.
⑤ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 20 min at 4℃.
⑥ Collect the filtrate and preserve 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% Mouse heart tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse kidney tissue homogenate	1
Mouse plasma	1
Rat serum	1
Rat plasma	1
1×10^6 CHO cells	1
1×10^6 Molt-4 cells	1
1×10^6 HL-60 cells	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

The sample peration to be tested on the ice box and should be tested within 4 hours.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the well.

Sample well: add 20 μL of sample into the well.

② Add 120 μL of substrate working solution into each well.

③ Add 70 μL of enzyme working solution into each well.

④ Add 50 μL of fluorescence probe into each well.

⑤ Mix fully with fluorescence microplate reader for 5s. Incubate at 37°C for 10 min and measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm. $\triangle F = F_{sample} - F_{blank}$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.
3. Plot the standard curve by using absoluted fluorescence 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 and plasma samples:

$$\text{Acetyl-CoA content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue sample:

$$\text{Acetyl-CoA content (μmol/kg wet weight)} = (\Delta F - b) \div a \div m \times v \times f$$

3. Cell sample:

$$\text{Acetyl-CoA content (nmol/10^6)} = (\Delta F - b) \div a \div n \times v \times f$$

[Note]

ΔF:$\Delta F = F_{sample} - F_{blank}$. ($F_{blank}$ is the fluorescence value when the standard concentration is 0).

m: The weight of tissue, kg.

v: The volume of extracting solution in the preparation of sample, L.

n: The number of cell sample/10^6.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.50	2.50	4.00
%CV	1.7	1.9	4.1

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.50	2.50	4.00
%CV	2.7	3.3	3.6

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 97.6%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	1.5	2.5	4
Observed Conc.(μmol/L)	1.4	2.4	4.3
Recovery rate (%)	92	94	107

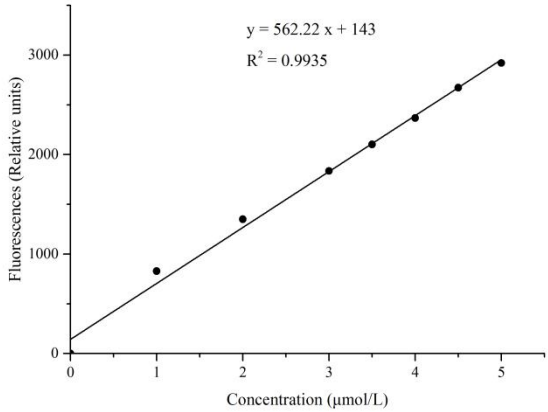
Sensitivity

The analytical sensitivity of the assay is 0.27 μmol/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 fluorescence 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 (μmol/L)	0	1	2	3	3.5	4	4.5	5
Fluorescence value	978	1810	2337	2789	3112	3360	3655	3935
	959	1783	2301	2816	3028	3310	3625	3842
Average fluorescence value	968	1797	2319	2803	3070	3335	3640	3888
Absoluted fluorescence value	0	829	1351	1835	2102	2367	2672	2920



Appendix Π Example Analysis

Example analysis：

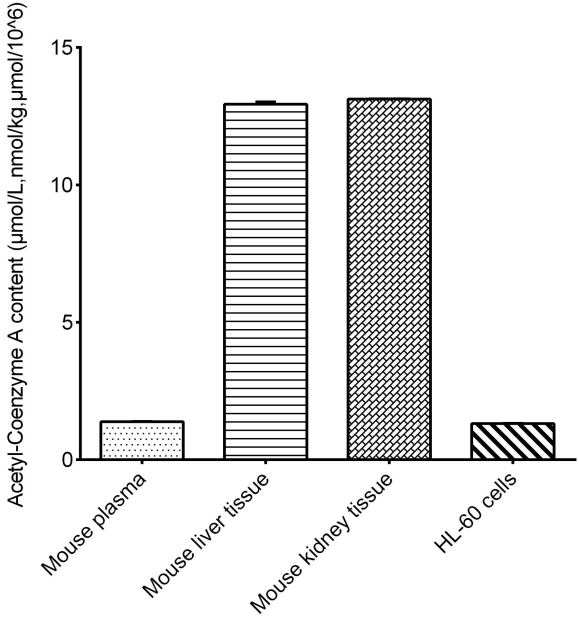
Take 20 μL of 10% mouse kidney tissue filtrate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 562.22x + 143, the average fluorescence value of the sample well is 1919.06, the average fluorescence value of the blank well is 968.10, ΔF = 1919.06 – 968.10 = 950.96, and the calculation result is:

Acetyl-CoA content (μmol/kg wet weight) =(950.96 - 143) ÷ 562.22 ÷ 0.1 × 0.9

= 12.93 μmol/kg wet weight

Detect mouse plasma, 10% mouse liver tissue homogenate, 10% mouse kidney tissue homogenate and 1×10^6 HL-60 cells 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.