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

Catalog No：E-BC-K907-M

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

Measuring instrument：Microplate reader(440-460 nm)

Detection range：0.005-0.5 mmol/L

Elabscience® Coenzyme A (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.

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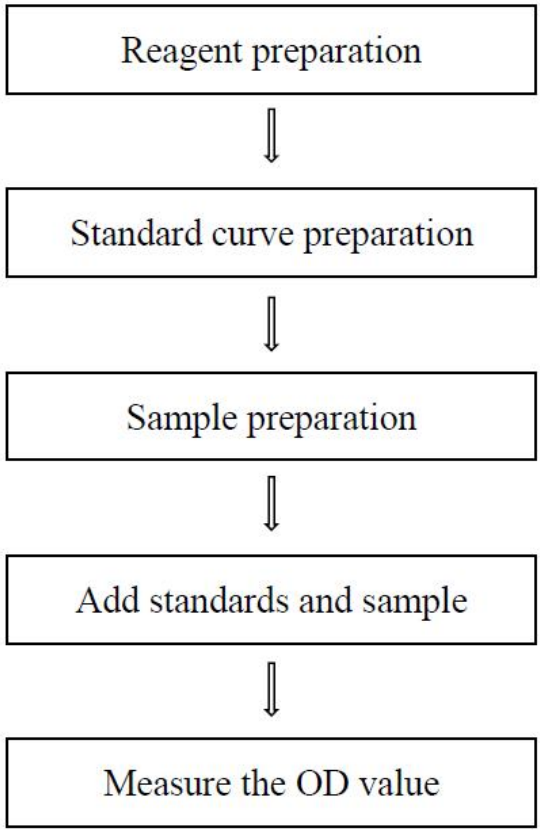
Assay summary



Intended use

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

Detection principle

Coenzyme A (CoA) is composed of units derived from cysteine, pantothenic acid, and ATP. It plays an important role in the synthesis and oxidation of fatty acids, pyruvate oxidation in the citric acid cycle, and other biological processes.

The enzymatic reaction of Coenzyme A produces NADH, and the chromogenic substance produced by reaction with the chromogenic agent has a characteristic absorption peak at 450 nm. The content of CoA in the sample can be determined by detecting the absorbance.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	50 mL × 1 vial	50 mL × 2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate	2.5 mL× 1 vial	5 mL× 1 vial	-20℃, 12 months, shading light
Reagent 4	Accelerant	0.8 mL× 1 vial	1.6 mL× 1 vial	-20℃, 12 months, shading light
Reagent 5	Enzyme Solution	0.5 mL× 1 vial	1.0 mL× 1 vial	-20℃, 12 months, shading light
Reagent 6	Chromogenic Agent	1.5 mL× 1 vial	1.5 mL× 2 vials	-20℃, 12 months, shading light
Reagent 7	Standard	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
	Microplate	48 wells	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 (440-460 nm, optimum wavelength: 450 nm), Incubator (37℃), Vortex mixer

Reagents:

1 mol/L Perchloric acid, 3 mol/L $KHCO_3$

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of reaction working solution:
For each well, prepare 130 μL of reaction working solution (mix well 90 μL of buffer solution, 30 μL of substrate and 10 μL of accelerant). Keep reaction working solution on ice during use. Store at -20°C for 3 days protected from light.

③ The preparation of enzyme working solution:
For each well, prepare 40 μL of enzyme working solution (mix well 8 μL of enzyme solution and 32 μL of buffer solution). The enzyme working solution should be prepared on spot, keep the prepared solution on ice during use and used it up within 1 day.

④ The preparation of 1 mmol/L standard solution:
Dissolve one vial of standard with 1 mL of buffer solution, mix well to dissolve. Keep the prepared solution on ice before using. Aliquoted storage at -20°C for 7 days, and avoid repeated freeze/thaw cycles is advised.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions

after use.

Dilute 1 mmol/L standard with buffer solution to a serial concentration, the recommended dilution gradient is 0, 0.05, 0.2, 0.25, 0.3, 0.4, 0.45, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.05**	**0.2**	**0.25**	**0.3**	**0.4**	**0.45**	**0.5**
1 mmol/L standard (μL)	0	10	40	50	60	80	90	100
Buffer solution (μL)	200	190	160	150	140	120	110	100

Sample preparation

① Sample preparation

Serum (plasma):

① Mix well 50 μL of serum or plasma samples and 100 μL of 1 mol/L perchloric acid for at least 1 min. Then add 3 mol/L $KHCO_3$ to adjust the pH to 7-8.

② Centrifuge at 10000×g for 10 min at 4°C, collect supernatant and keep it for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL of extracting solution with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it for detection.

④ Please prepare sufficient sample according to the test wells. For example, mix well 80 μL of supernatant and 20 μL 1 mol/L perchloric acid for at least 1 min. Then add 3 mol/L $KHCO_3$ to adjust the pH to 7-8 (add $KHCO_3$ to adjust pH to produce intense gas, please be careful to add slowly).

⑤ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep 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 Homogenize 1×10^6 cells in 200 μL extracting solution with a ultrasonic cell disruptor at 4℃.

③ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it for detection.

④ Please prepare sufficient sample according to the test wells. For example, mix

well 40 μL of supernatant and 20 μL 1 mol/L perchloric acid for at least 1 min. Then add 3 mol/L $KHCO_3$ to adjust the pH to 7-8 (add $KHCO_3$ to adjust pH to produce intense gas, please be careful to add slowly).

⑤ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

Note: In the process of the sample preparation with acid and alikali, the sample is diluted. According to the volume of perchloric acid and $KHCO_3$, calculate the sample dilution factor.

② Dilution of sample

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

Sample type	Dilution factor
Human serum (plasma)	1
Mouse serum (plasma)	1
10% Mouse lung tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse brain tissue homogenate	1
1×10^6 293T cell homogenate	1
1×10^6 Molt-4 cell homogenate	1
1×10^6 HL-60 cell 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

During the preparation, keep the sample on ice, and detect it within 4 hours.

Operating steps

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

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

② Add 130 μL of reaction working solution into each well.

③ Add 40 μL of enzyme working solution into each well.

④ Add 20 μL of chromogenic agent into each well.

⑤ Mix fully with microplate reader for 3 s, incubate at 37°C for 30 min. Measure the OD value of each well at 450 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:

1. **Serum (plasma) samples:**

$$\text{CoA content (mmol/L)} = (\Delta A_{450} - b) \div a \times f$$

2. **Tissue sample:**

$$\text{CoA content (mmol/kg wet weight)} = (\Delta A_{450} - b) \div a \div m \times V \times f$$

3. **Cell samples:**

$$\text{CoA content (mmol/10^6)} = (\Delta A_{450} - b) \div a \div n \times V \times f$$

[Note]

ΔA_{450}: $\triangle A = A_{sample} - A_{blank}$ (the OD value when the standard concentration is 0).

m: The weight of the sample, kg.

V: The volume of the added extracting solution, L.

n: The number of the cell samples, 10^6.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	0.15	0.30	0.45
%CV	0.8	1.9	2.2

Inter-assay Precision

Three human serum 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)	0.15	0.30	0.45
%CV	0.6	3.3	7.5

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 99.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.3	0.45
Observed Conc. (mmol/L)	0.1	0.3	0.5
recovery rate(%)	90	98	110

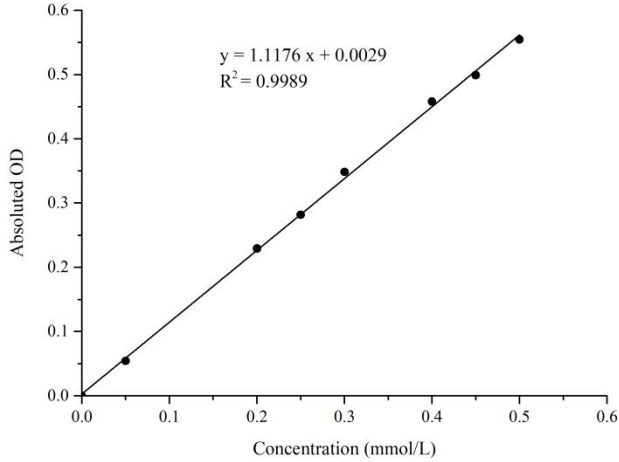
Sensitivity

The analytical sensitivity of the assay is 0.005 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**	**0.05**	**0.2**	**0.25**	**0.3**	**0.4**	**0.45**	**0.5**
OD	0.080	0.134	0.313	0.363	0.428	0.540	0.576	0.634
	0.080	0.134	0.306	0.361	0.429	0.536	0.582	0.635
Average OD	0.080	0.135	0.310	0.362	0.429	0.538	0.579	0.635
Absoluted OD	0.000	0.055	0.230	0.282	0.349	0.458	0.499	0.555



Appendix Π Example Analysis

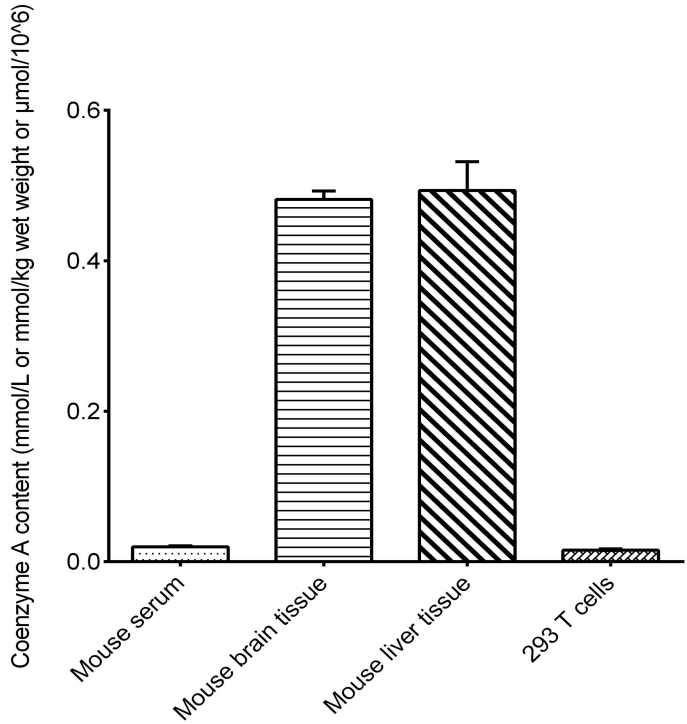
Example analysis：

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

Standard curve: y = 1.1176 x + 0.0029, the average OD value of the sample well is 0.144, the average OD value of the blank well is 0.080, $\triangle A_{450}$ = 0.144 – 0.080 = 0.064， and the calculation result is:

CoA content (mmol/kg wet weight) = (0.064 - 0.0029) ÷ 1.1176 ÷ 0.0001 × 0.0009 = 0.492 mmol/kg wet weight

Detect mouse serum, 10% mouse brain tissue homogenate, 10% mouse liver tissue homogenate and 1×10^6 293T 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.