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

Catalog No：E-BC-K885-M

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

Measuring instrument：Microplate reader(600-640 nm)

Detection range：0.016-1 mg/mL

Elabscience® Coenzyme Q10 (CoQ10) 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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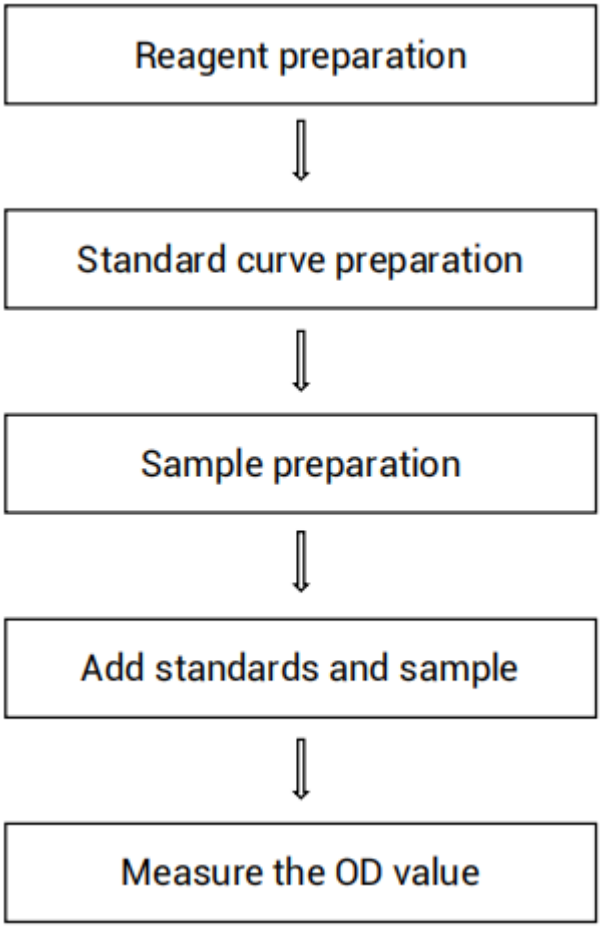
Assay summary



Intended use

This kit can be used to measure coenzyme Q10 (CoQ10) content in animal tissue samples.

Detection principle

Coenzyme Q10 (Coenzyme Q10, CoQ10) is a lipid-soluble antioxidant that plays a significant role in energy metabolism, antioxidation, cell membrane stability, cardiovascular health, and anti-aging.
After the sample was extracted with organic reagents, CoQ10 developed color with the chromogenic reagent under alkaline conditions and had a maximum absorption peak at 620 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution A	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Extraction Solution B	45 mL × 1 vial	45 mL × 2 vials	-20°C, 12 months, shading light
Reagent 3	Chromogenic Agent A	18 mL × 1 vial	36 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent B	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Standard	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	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:

Microplate reader (600-640 nm, optimum wavelength: 620 nm), Incubator, Homogenizer, Fume hood, Centrifuge

Reagent preparation

① Equilibrate all the reagents to 25°C before use in a fume hood.

② Mix the chromogenic agent B fully before use.

③ The preparation of chromogenic working solution:
For each well, prepare 450 μL of chromogenic working solution (mix well 300 μL of chromogenic agent A and 150 μL of chromogenic agent B). The chromogenic working solution should be prepared on spot protected from light and used up within 8 h.

④ The preparation of 2 mg/mL standard solution:
Dissolve one vial of standard with 5 mL of extraction solution B, mix well to dissolve. Store at -20°C for 3 days protected from light.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 2 mg/mL standard solution with extraction solution B to a serial concentration, the recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.05**	**0.1**	**0.2**	**0.4**	**0.6**	**0.8**	**1**
2 mg/mL standard (μL)	0	12.5	25	50	100	150	200	250
Extraction solution B (μL)	500	487.5	475	450	400	350	300	250

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.1 g).

② Homogenize 0.1 g tissue in 0.4 mL extraction solution A and 0.6 mL extraction solution B with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 5 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice protected from light for detection. The supernatant should be used up within 8 h.

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1

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

The key points of the assay

① The reagent contains volatile organic solvents. All operations should be carried out in a fume hood.

② To prevent the loss of reagents due to evaporation, all reagents should have their caps tightened as soon as possible after use.

③ It's better to measure no more than 30 wells (including standard wells) at same time.

④ Chromogenic Agent B should be mixed thoroughly before use to avoid a decrease in the overall measured value.

Operating steps

① Standard tube: Add 50 μL of standard solution with different concentrations into the tubes.
Sample tube: Add 50 μL of samples into tubes.

② Add 450 μL of chromogenic working solution into each tube.

③ Mix fully for 30 s and centrifuge at 500×g for 1 min at 4°C. Incubate at 37°C for 10 min.

④ Take 300 μL supernatant of each tube to the microplate wells.
Measure the OD value of each well at 620 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:

Tissue samples:

$$\underset{\text{(mg/g wet weight)}}{\text{CoQ10}} = \frac{\Delta A - b}{a} \div \frac{m}{V} \times f$$

[Note]

ΔA: $\Delta A = OD_{sample} - OD_{blank}$.

m: The weight of sample, g.

V: The volume of extraction solution B in the preparation step of sample, mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse heart 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 (mg/mL)	0.25	0.55	0.85
%CV	5.1	6.7	5.2

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.25	0.55	0.85
%CV	9.5	6.3	6.5

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/mL)	0.250	0.550	0.850
Observed Conc. (mg/mL)	0.242	0.580	0.875
Recovery rate (%)	97	105	103

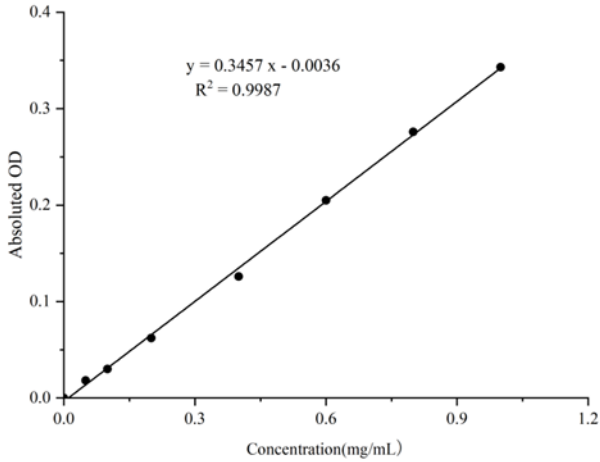
Sensitivity

The analytical sensitivity of the assay is 0.016 mg/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.

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 (mg/mL)	0	0.05	0.1	0.2	0.4	0.6	0.8	1
OD value	0.038	0.056	0.068	0.099	0.163	0.233	0.312	0.375
	0.039	0.056	0.069	0.101	0.165	0.253	0.317	0.388
Average OD value	0.039	0.056	0.069	0.100	0.164	0.243	0.315	0.382
Absolute OD value	0.000	0.018	0.030	0.062	0.126	0.205	0.276	0.343



Appendix Π Example Analysis

Example analysis：

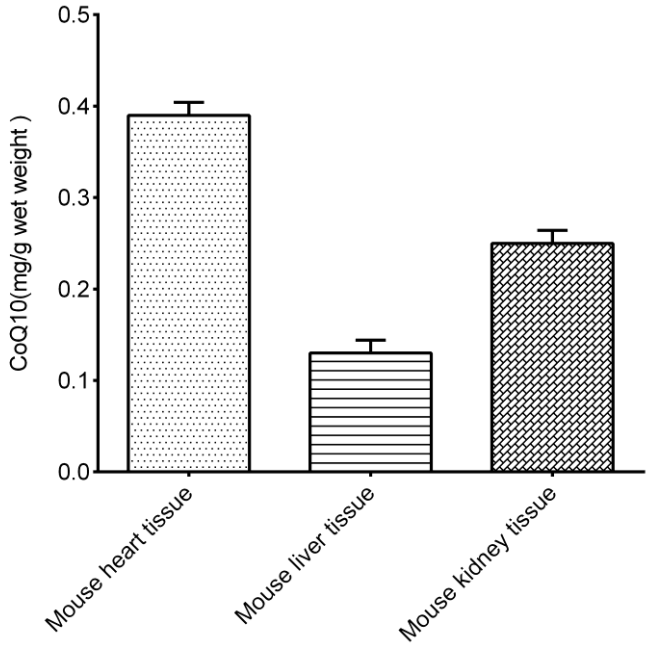
Take 50 μL of 10% mouse heart tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.3457 x – 0.0036, the OD value of the blank well is 0.038, the OD value of the sample well is 0.057. The calculation result is:

CoQ10 (mg/g wet weight) = (0.057 - 0.038 + 0.0036) ÷ 0.3457 ÷ (0.1 ÷ 0.6)

= 0.39 mg/g wet weight

Detect 10% mouse heart tissue homogenate, 10% mouse liver tissue homogenate and 10% mouse 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.