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

Catalog No：E-BC-K1107-M

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

Measuring instrument：Microplate reader(412 nm)

Detection range：2.60-155.7 U/L

Elabscience® Carnitine Palmitoyl Transferase-I (CPT-I) Activity 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

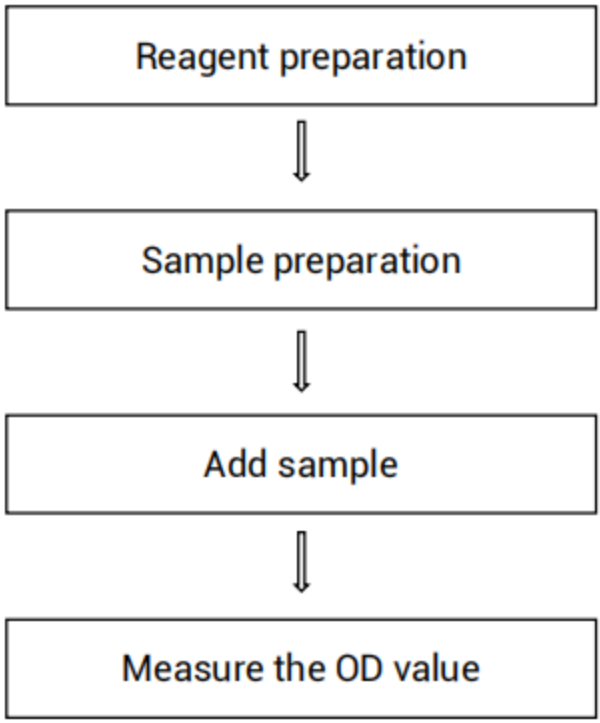
Email: techsupport@elabscience.com

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



Intended use

This kit can be used to measure carnitine palmitoyl transferase-I (CPT-I) activity in animal tissue and cell samples.

Detection principle

Carnitine Palmitoyl Transferase-I (CPT-I) is a key rate-limiting enzyme in the fatty acid β-oxidation process. Its mechanism of action is closely related to its core function in fatty acid β-oxidation, involving substrate recognition, acyl transfer, and the synergistic effect of regulatory factors. CPT-I catalyzes the formation of acyl-carnitine from fatty acyl-CoA, and simultaneously reacts with a chromogenic agent to form TNB, which causes an increase in absorbance at 412 nm. The activity of CPT-I can be determined by measuring the rate of the increase in absorbance at 412 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Extraction Solution A	55 mL × 1 vial	55 mL × 2 vials	-20℃, 12 months
Reagent 2	Extraction Solution B	24 mL × 1 vial	48 mL × 1 vial	-20℃, 12 months
Reagent 3	Substrate A	6 mL × 1 vial	12 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Substrate B	6 mL × 1 vial	12 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Chromogenic Agent	2 mL × 1 vial	4 mL × 1 vial	-20℃, 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 (412 nm), Incubator, Vortex, Homogenizer, Centrifuge, Ultrasonic disintegrator

Reagent preparation

Equilibrate all the reagents to 25°C before use.

Sample preparation

① Sample preparation

Tissue samples:

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

② Homogenize 20 mg tissue in 200 μL extraction solution A with a dounce homogenizer at 4 °C.

③ Centrifuge at 600 × g at 4 °C for 5 min to remove insoluble material. Transfer the supernatant to a new EP tube (determine the protein concentration of supernatant).

④ Centrifuge at 12000 × g at 4°C for 10 min. Take the sediment and add 0.2 mL of extraction solution B.

⑤ Sonicate for 5 min, centrifuge at 4°C for 10 min at 12000 × g. Take the supernatant and place it on ice for detection and detect within 8 h.

⑥ Meanwhile, determine the protein concentration of supernatant (E-BC-K318- M).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^7 cells).

② Homogenize 1×10^7 cells in 1 mL extraction solution A with a ultrasonic cell disruptor at 4°C.

③ Centrifuge at 600 × g at 4 °C for 5 min to remove insoluble material. Transfer the supernatant to a new EP tube (determine the protein concentration of supernatant).

④ Centrifuge at 12000 × g at 4°C for 10 min. Take the sediment from the high-speed centrifuge, add 0.2 mL of extraction solution B.

⑤ Sonicate for 5 min, centrifuge at 4°C for 10 min at 12000 × g. Take the supernatant and place it on ice for detection and detect within 8 h.

⑥ Meanwhile, determine the protein concentration of supernatant (E-BC-K318- M).

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	1-2
10% Mouse kindey tissue homogenate	1-2
1×10^7 A549 cells	1
1×10^7 293T cells	1
1×10^7 HL60 cells	1
1×10^7 K562 cells	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 CPT-I activity of cell samples is usually relatively low. The incubation time can be extended from 5-10 min when detect. Correspondingly, the reaction time in the calculation formula should be modified to 10 min.

② If the ΔA value of sample well is greater than 0.3, the sample should be diluted before detection.

③ Each experiment should not exceed 20 wells.

Operating steps

① Sample well: Add 10 μL of sample into the wells.

② Add 100 μL of substrate A into sample wells.

③ Add 100 μL of substrate B into sample wells.

④ Add 30 μL of chromogenic agent into sample wells.

⑤ Mix fully and measure the OD value of each well at 412 nm as A_1.

⑥ Incubate at 37°C for 5 min immediately.

⑦ Mix fully and measure the OD value of each well at 412 nm as A_2, $\Delta A = A_2 - A_1$.

Calculation

The tissue or cell sample:

Definition: The amount of enzyme in 1 g animal tissue protein or cell protein per minute that catalyze substrate to product 1 μmol TNB at 37°C is defined as 1 unit.

$$\text{CPT-I activity (U/gprot)} = \frac{\Delta A \times V_2}{\varepsilon \times d} \times 10\text{^}6^{*} \div T \div (C_{pr} \times V_1) \times f$$

$$= \Delta A \times 519^{*} \div C_{pr} \times f$$

[Note]

ΔA: A_2-A_1

ε: The molar extinction coefficient of TNB at 412 nm, 13600 L/moL/cm.

d: Optical path, 0.68 cm.

10^6*:1 mol = 1×10^6 μmol.

V_1: The volume of sample, 10 μL = 10^-5 L.

V_2: The volume of reaction system, 240 μL = 2.4 × 10^-4 L.

C_{pr}: Concentration of protein in sample, gprot/L.

T: Reaction time, 5 mim.

f: Dilution factor of sample before tested.

519*: Simplified value.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 1×10^7 A549 cell 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 (U/L)	4.7	9.5	13.8
%CV	3.8	2.7	3.2

Inter-assay Precision

Three 1×10^7 A549 cell samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	37.1	47.5	58.1
%CV	9.6	6.5	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 100.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	7.4	14.0	24.7
Observed Conc. (U/L)	7.2	14.2	25.5
Recovery rate (%)	97	101	103

Sensitivity

The analytical sensitivity of the assay is 2.60 U/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.

Appendix Π Example Analysis

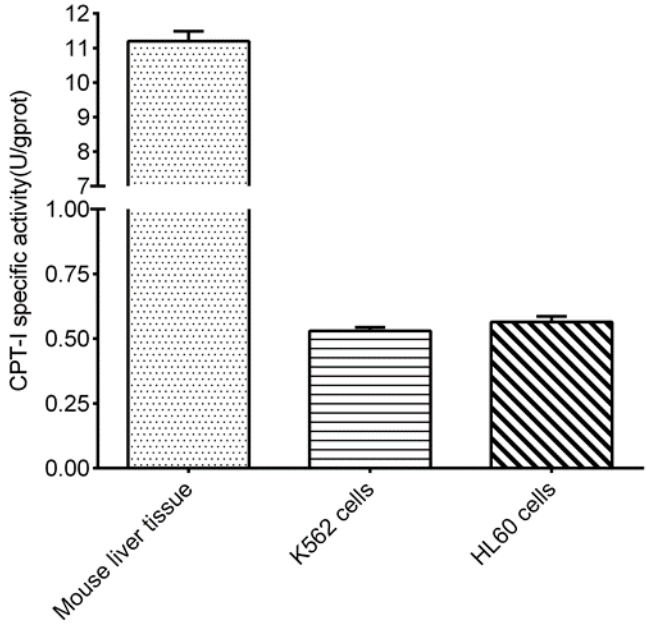
Example analysis：

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

The A_1 value of the sample well is 0.349, the A_2 value of the sample well is 0.639, the concentration of protein in sample is 13.2 gprot/L and the calculation result is:

CPT-I activiy（U/gprot）= （0.639 - 0.349） × 519 ÷ 13.2 = 11.40 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 13.2 gprot/L), 9.8×10^7 K562 cells (the concentration of protein is 14.0 gprot/L), 11.1×10^7 HL60 cells (the concentration of protein is 16.4 gprot/L) 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.