Human CPT1A (Carnitine O-palmitoyltransferase 1, liver isoform) ELISA Kit

Article Number EKX-YQCKDW-96

User Manual

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@nordicbiosite.com

Nordic Biosite
Part of Europa Biosite

Table of Contents

General information 3
Kit Components 3
Typical Data & Standard Curve 4
Specificity 4
Recovery 5
Linearity 5
Precision 5
Stability 5
Principle of the Assay 6
Precautions for Use 6
Material Required But Not Supplied 6
Washing 7
Sample Collection and Storage (universal) 7
Sample Dilution Guideline 9
Reagent Preparation and Storage 9
Assay Procedure 10

General information

Size: 96T
Reactivity: Human
Range: 15.625-1000pg/ml
Sensitivity: 9.375pg/ml
Application: For quantitative detection of CPT1A in serum, plasma, tissue homogenates and other biological fluids.
Storage: 4°C for 6 months
Principle: Sandwich

NOTE: FOR RESEARCH USE ONLY.

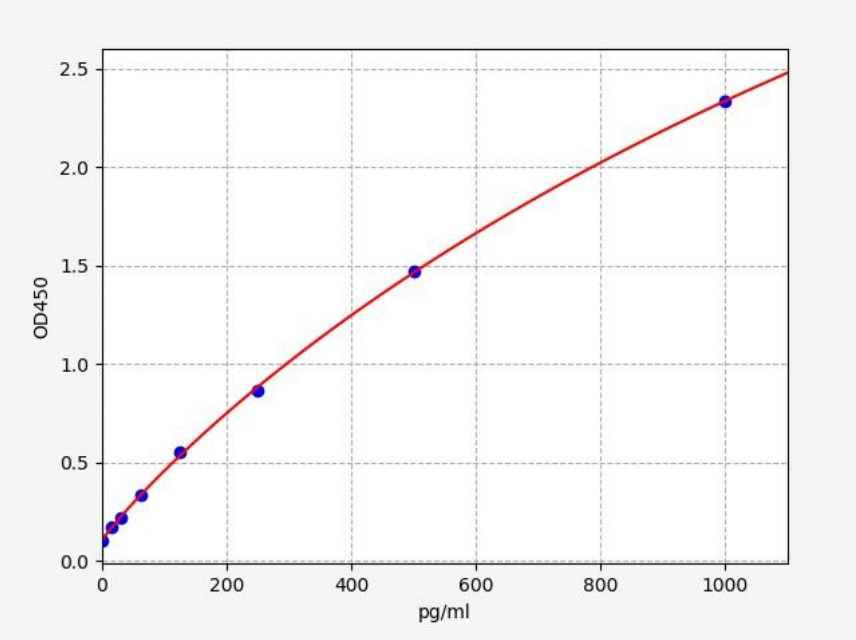
Kit Components

Item	Specifications (96T)	Storage
Micro ELISA Plate (Dismountable)	8 ×12	4℃/-20℃
Lyophilized Standard	2 vials	4℃/-20℃
Sample / Standard dilution buffer	20 ml	4℃
Biotin-detection antibody (Concentrated)	120 µl	4℃ (Avoid Direct Light)
Antibody dilution buffer	10 ml	4℃
HRP-Streptavidin Conjugate (SABC)	120 µl	4℃ (Avoid Direct Light)
SABC dilution buffer	10 ml	4℃
TMB substrate	10 ml	4℃ (Avoid Direct Light)
Stop solution	10 ml	4℃
Wash buffer (25X)	30 ml	4℃
Plate Sealer	5 items	
User Manual	1 copy	

Typical Data & Standard Curve

Results of a typical standard operation of a CPT1A ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves. (N/A=not applicable)
NOTE: For reference only.

STD (ng/ml)	OD-1	OD-2	Average	Corrected
0	0.103	0.107	0.105	0
15.625	0.170	0.174	0.172	0.067
31.25	0.214	0.220	0.217	0.112
62.5	0.328	0.338	0.333	0.228
125	0.548	0.564	0.556	0.451
250	0.851	0.875	0.863	0.758
500	1.451	1.493	1.472	1.367
1000	2.303	2.369	2.336	2.231



Specificity

This assay has high sensitivity and excellent specificity for detection of CPT1A. No significant cross-reactivity or interference between CPT1A and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between CPT1A and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with certain level of CPT1A and the recovery rates were calculated by comparing the measured value to the expected amount of CPT1A in samples.

Matrix	Recovery range (%)	Average (%)
Serum (n=5)	85-105	93
EDTA plasma (n=5)	93-105	100
Heparin plasma (n=5)	87-104	99

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of CPT1A and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum (n=5)	85-101%	88-101%	87-99%
EDTA plasma (n=5)	87-103%	87-105%	81-95%
Heparin plasma (n=5)	90-105%	83-94%	84-91%

Precision

Intra-Assay: CV<8%
Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard (n=5)	37°C for 1 months	4°C for 6 months
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same person from the beginning to the end.

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96-well plates and the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently and later washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalysed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. After reading the O.D. absorbance at 450nm in a microplate reader, the concentration of the target can be calculated.

Precautions for Use

1. To inspect the validity of experiment operation and to ensure the correct sample dilution, a pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep the plate dry.
3. Before using the kit, briefly spin the tubes with the components.
4. Store TMB reagents protected from light.
5. Washing process is important. Incomplete wash may easily cause false positive result and high background.
6. Duplicates are recommended for both standard and sample testing.
7. Do not let the microplate dry during the assay. A dry plate will inactivate active components on the plate.
8. Do not reuse tips and tubes to avoid cross contamination.
9. Avoid using reagents from different batches together.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required But Not Supplied

1. Microplate reader (wavelength: 450 nm)
2. 37℃ incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Washing

Manual: Discard the solution in the plate without touching the side walls. Gently tap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 min, then aspirate contents from the plate, and tap again the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350µl wash buffer. After the final wash, invert the plate, and gently tap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer is set for soaking 1 min. (Note: set the height of the needles; be sure the fluid can be sipped up completely)

Sample Collection and Storage (universal)

- Serum: Place whole blood sample at room temperature for 2 hours or leave it at 4°C over-night and centrifuge for 20 min at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- Plasma: Collect plasma using (EDTA-Na2 or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2 - 8°C within 30 min of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- Tissue Homogenates: As hemolysis affects the result of the assay, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince the tissue after weighing it and homogenize it in PBS (the volume depends on the weight of the tissue). Normally, 9mL PBS would be appropriate to 1 g of tissue sample. It is recommended to add protease inhibitors into the PBS) with a glass homogenizer on ice. To further break the cells, we recommend to sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates should then be centrifuged for 5min at 5000×g to get the supernatant. The total protein concentration is determined by a BCA assay and the total protein concentration in each well should not exceed 0.3mg.
- Cell Culture Supernatant: Centrifuge supernatant for 20 minutes at 1000×g at 2 - 8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
- Adherent and Suspension Cell Culture: Use three T25 flasks or one T75 flask for cell culture, the number of cells (1x107).

1. Suspension cell: centrifuge at 2500 rpm at 2-8℃ for 5 minutes; collect clarified cell culture supernatant;
2. Adherent cell: collect supernatant directly; centrifuge at 2500 rpm at 2-8℃ for 5 minutes; collect clarified cell culture supernatant for immediate detection or store it separately at -80℃.

- Cell Lysate Preparation: Two types of cell lysates are specified below:
  1. Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes; Then add pre-cooling PBS into collected cell and gently mix. Recollect cell by repeating centrifugation. Add 0.5-1ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant is not recommended due to the interfering with antigen-antibody reaction). Add suitable protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on ice for 30min-1h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Alternatively, cells are subject to fragmentation by ultrasonic cell disruptor (300W, 3~5 s/time, 30s intervals, four-five times) or ultrasonic generator (14μm for 30s). At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8℃ for 10 minutes. Then, the supernatant is added into EP tube and stored at -80℃.
  2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS once. Then, add 0.5-1ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant is not recommended due to the interfering with antigen-antibody reaction). Add the suitable protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape adherent cell gently with a cell scraper. Add the cell suspension into centrifugal tube. Lyse the cell on ice for 30min-1h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Alternatively, cells are subject to fragmentation by ultrasonic generator (14μm for 30s) or ultrasonic cell disruptor (300W, 3~5 s/time, 30s intervals, four-five times). At the end of lysate/ultrasonic disruption, centrifuge at 10000rpm at 2-8℃ for 10 minutes. Then, the supernatant is added into EP tube and stored at -80℃.
- Other Biological Fluids: Centrifuge samples for 20 min at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days should be stored at 4°C. Otherwise, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

Sample Dilution Guideline

The user should estimate the concentration of target protein in the test sample and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer. Several trials may be necessary. The test sample must be well mixed with the dilution buffer. Both standard curves and sample should be included in pilot experiment. In case of samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

The matrix components in the sample will affect the test results, therefore the sample needs to be diluted at least 1:2 with Sample Dilution Buffer before testing!

Reagent Preparation and Storage

Bring all reagents to room temperature for 20 min before use.

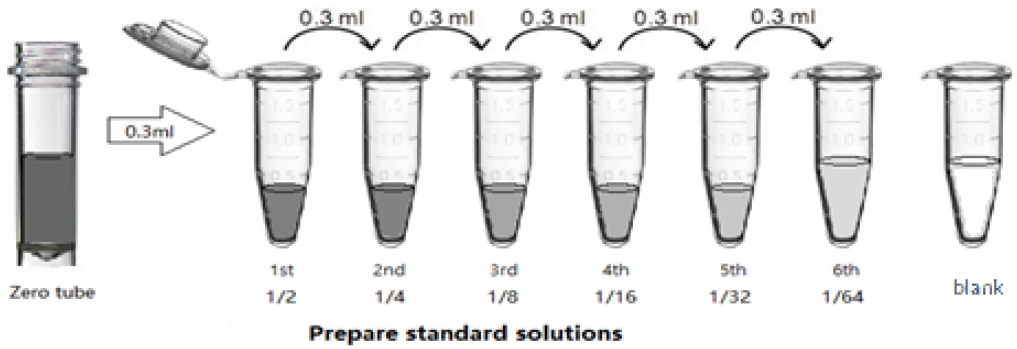
1. Wash Buffer

If crystals have formed in the concentrate, warm it in 40°C water bath (heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.
Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Leave the rest of the solution at 4°C.

2. Standard

1). Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 min and mix thoroughly.
Note: If the standard tube concentration is higher than the range of the kit, please dilute it and label it as a zero tube.

2). Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 0.3ml of the above Standard solution (from zero tube) into 1st tube and mix thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



Note: It is recommended to use Standard Solutions within 2 hours.

3. Preparation of Biotin-labeled Antibody Working Solution
Prepare it within 1 hour before experiment.
1) Calculate required total volume of the working solution: 100ul/well x quantity of wells. Allow 0.1-0.2 ml more than the total volume.
2) Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl Biotin-labelled antibody into 99μl Antibody Dilution Buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:
Prepare it within 30 minutes before experiment.
1). Calculate required total volume of the working solution: 0.1ml / well × quantity of wells (allow 0.1-0.2ml more than the total volume).
2). Dilute the SABC with SABC Dilution Buffer at 1:100 and mix thoroughly (i.e. add 1μl of SABC into 99μl of SABC Dilution Buffer).

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C. It is recommended to plot a standard curve for each test.

1. Set standard, **test samples (diluted at least 1:2 with Sample Dilution Buffer), control (blank)** wells on the pre-coated plate respectively, and then record their positions. It is recommended to measure each standard and sample in duplicate.
2. **Prepare Standards:** Aliquot 100μl of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube and Sample Dilution Buffer (blank) into the standard wells.
3. **Add Samples:** Add 100μl of properly diluted sample into test sample wells.

4. Incubate: Seal the plate with a cover and incubate at 37°C for 90 min.
5. Wash: Remove the cover and discard the plate content. Wash the plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
6. Biotin-labeled Antibody: Add 100µl Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 min.
7. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 min each time.
8. HRP-Streptavidin Conjugate (SABC): Add 100µl of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 min.
9. Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 min each time.
10. TMB Substrate: Add 90µl TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 min. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30min. You can terminate the reaction when apparent gradient appeared in standard wells.)
11. Stop: Add 50µl Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be the same as for the TMB Substrate Solution.
12. OD Measurement: Read the O.D. absorbance at 450 nm in Microplate Reader immediately after adding the stop solution.

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve may be plotted as the O.D.450 of each standard solution (Y) vs. the corresponding concentration of the standard solution (X). The target concentration of the samples may be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before the dilution.

Nordic Biosite
Part of Europa Biosite