Version 10-09/22

User's Manual

Human ETFDH (Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial) ELISA Kit

REF **DEIA-FN460**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For quantitative detection of Human ETFDH (Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial) in serum, plasma, tissue homogenates and other biological fluids.

Principles of Testing

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for ETFDH has been coated onto the wells of the microtiter strips provided. Standards and samples are pipetted into the wells and any ETFDH present is bound by the immobilized antibody. During the first incubation, the standards or samples and a biotinylated antibody specific for ETFDH are simultaneously incubated. After washing, the enzyme Streptavidin-HRP, that binds the biotinylated antibody is added, incubated and washed. A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of ETFDH present in the samples.

Reagents And Materials Provided

1. 96-well strip plate (Dismountable), 1 plate
2. Lyophilized Standard, 2 vials
3. Sample/Standard dilution buffer, 20 mL
4. Biotin-detection antibody (Concentrated), 120 uL
5. Antibody dilution buffer, 10 mL
6. HRP-Streptavidin Conjugate(SABC), 120 uL
7. SABC dilution buffer, 10 mL
8. TMB substrate, 10 mL
9. Stop solution, 10 mL
10. Wash buffer (25X), 30 mL
11. Plate Sealer, 5 pieces
12. Product Manual, 1 copy

Materials Required But Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.

- 37°C incubator.

- Precision pipettes to deliver 2 µL to 1 mL volumes.

- Adjustable 1-25 mL pipettes for reagent preparation.

- Distilled or deionized water.

- Log-log graph paper or computer and software for ELISA data analysis.

- Tubes to prepare standard or sample dilutions.

Storage

Store the unopened product at 2 - 8 °C. Do not use past expiration date.

Specimen Collection And Preparation

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes 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 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue homogenates: For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01 M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to get the supernate.

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernate and carry out the assay immediately.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.

Sample preparation: Samples should be clear and transparent and be centrifuged to remove suspended solids.

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80 °C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Reagent Preparation

Bring all kit components and samples to room temperature (20 - 25°C) before use.

1.Samples

Please predict the concentration before assaying. If concentrations are unknown or not within the detection range, a preliminary experiment is recommended to determine the optimal dilution.

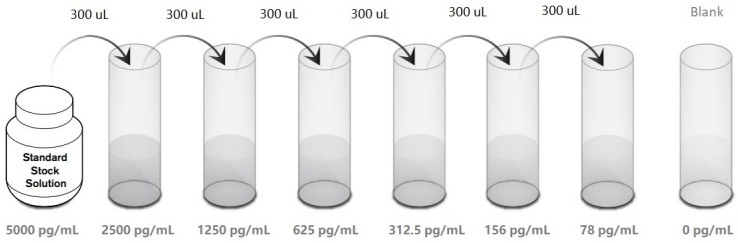
2. Wash Solution

If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Solution concentrate (25×) with 720 mL of deionized or distilled water to prepare 750 mL of Wash Solution (1×).

3. Standard

Add 1 ml of Sample / Standard dilution buffer into one Standard tube, This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Pipette 300 µL of the Sample / Standard dilution buffer into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer.



Note: The standard solutions are best used within 2 hours.

4. Preparation of Biotin-detection Antibody working solution

Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly within 1 hour of use. (i.e. Add 1 µL of Biotin-detection antibody into 99 µL of Antibody dilution buffer.)

5. Preparation of HRP-Streptavidin Conjugate (SABC) working solution

Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly within 30 minutes of use. (i.e. Add 1 µ L of SABC into 99 µL of SABC dilution buffer.)

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. **Wash plate 2 times before adding standard, sample and control (zero) wells.**
2. Add 100 µL of standard solutions, properly diluted sample, or control per well. Cover with the adhesive strip provided. Incubate for 90 mins at 37 °C.
3. Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of Biotin-detection antibody working solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
5. Seal the plate with a cover and incubate at 37°C for 60 min.
6. Remove the cover, and wash plate 3 times with Wash buffer as in step 3.

7. Add 100 µL of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
8. Remove the cover and wash plate 5 times with Wash buffer as in step 3, and each time let the wash buffer stay in the wells for 1-2 min.
9. Add 90 µL of TMB substrate into each well, Incubate for 15 minutes at room temperature. **Protect from light.**
10. Add 50 µL of Stop Solution into each well and mix thoroughly. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

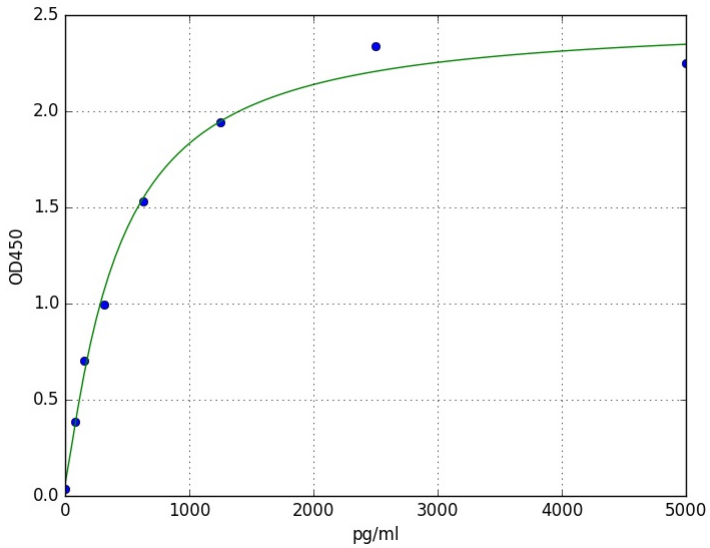
Calculation

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

For calculation, (the relative O.D.450) = (the O.D.450 of each well)–(the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be interpolated from the standard curve.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve



Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Range

78-5000 pg/mL

Sensitivity

46.9 pg/mL

Specificity

This assay has high sensitivity and excellent specificity for detection of ETFDH . No significant cross-reactivity or interference between Pola2 and analogues was observed.

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

Linearity

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

Sample	1:2	1:4	1:8	1:16
serum(n=5)	86-98%	92-103%	90-103%	85-104%
EDTA plasma(n=5)	86-96%	89-100%	83-101%	85-92%
heparin plasma(n=5)	93-98%	80-89%	87-100%	80-99%

Recovery

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

Matrix	Recovery range(%)	Average(%)
serum(n=5)	90-104	96
EDTA plasma(n=5)	86-104	97
heparin plasma(n=5)	87-96	92

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected

from light

3. Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
4. Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
5. Reagents from different batches should not be mixed.
6. Use thoroughly clean glassware
7. We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
8. All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.