Version 22-04/24

User's Manual

DPD(Deoxypyridinoline) ELISA Kit

REF **DEIANS015**

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

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PRODUCT INFORMATION

Intended Use

For quantitative detection of DPD in serum, plasma, tissue homogenates and other biological fluids.

Principles of Testing

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with antibody. During the reaction, target in the sample or standard competes with a fixed amount of Biotin-Antigen. Excess conjugate and unbound sample or standard are washed from the plate. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. The concentration of target in the samples is then determined by comparing the OD of the samples to the standard curve.

Reagents And Materials Provided

No.	Item	Specifications(96T)	Storage
E001	ELISA Microplate(Dismountable)	8×12	2-8°C/-20°C
E002	Lyophilized Standard	2vial	2-8°C/-20°C
E039	Sample Dilution Buffer	20ml	2-8°C
E003	Biotin-labeled Antigen(Concentrated)	60ul	2-8°C(Avoid Direct Light)
E040	Antigen Dilution Buffer	10ml	2-8°C
E034	HRP-Streptavidin Conjugate(SABC)	120ul	2-8°C(Avoid Direct Light)
E049	SABC Dilution Buffer	10ml	2-8°C
E024	TMB Substrate	10ml	2-8°C(Avoid Direct Light)
E026	Stop Solution	10ml	2-8°C
E038	Wash Buffer(25X)	30ml	2-8°C
E006	Plate Sealer	5pieces	
E007	Product Description	1copy	

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C 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

Storage

2-8°C for 6 months

Specimen Collection And Preparation

1. Serum: Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and 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.

2. Plasma: Collect plasma using EDTA-Na_2 or heparin 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.

3. Tissue Homogenates: As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Normal, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are 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 centrifuged for 5 minutes at 5000×g to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3mg.

4. Adherent and Suspension Cell Culture: Use three T25 flasks or one T75 flask for cell culture, the number of cells (1×10^7);

a. Suspension cell: centrifuge at 2500 rpm at 2-8°C for 5 minutes; collect clarified cell culture supernatant;

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

5. Cell Lysate Preparation: Two types of cell lysates are specified below.

a. Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8°C 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°C for 10 minutes. Then, the supernatant is added into EP tube and stored at -80°C.

b. 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°C for 10 minutes. Then, the supernatant is added into EP tube and stored at -80°C.

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

Note: Samples used within 5 days can be stored at 2-8°C; otherwise, they must be stored at -20°C or -80°C or liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test.

7. Sample Dilution

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, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

Reagent Preparation

Bring all reagents and samples to room temperature for 20 minutes before use.

1. Wash Buffer:

If crystals have formed in the concentrate, you can warm it with 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 to 750ml Wash Buffer with deionized or distilled water (The recommended resistivity of deionized or distilled water is 18MΩ). Put unused solution back at 2-8°C.

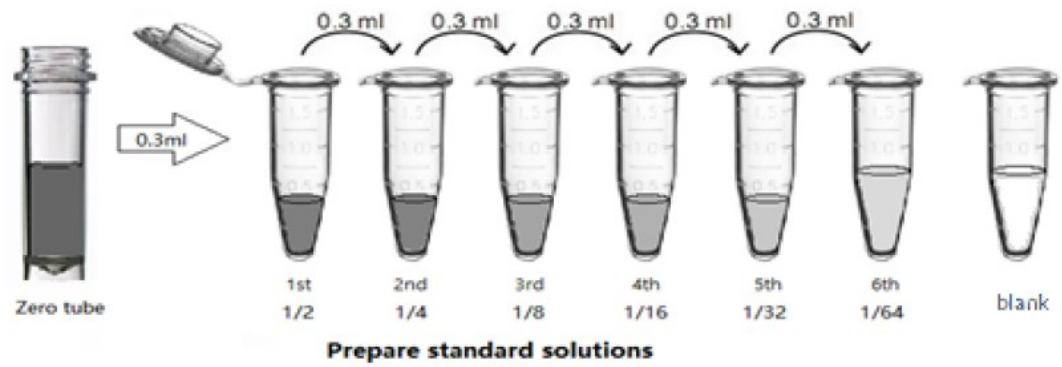
2. Biotin-labeled Antigen Working Solution:

Dilute the Biotin-labeled Antigen with Antigen Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl of Biotin-labeled Antigen into 99μl of Antigen Dilution Buffer.)

3. Standards:

a. Add 0.5 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly. **Note: If the standard tube concentration higher than the range of the kit,please dilute it and labeled as zero tube.**

b. 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 them thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2 nd tube to 3rd tube and mix them thoroug hly, and so on. Sample Dilution Buffer was used for the blank control. **Note: It is best to use Standard Solutions within 2 hours.**



4. Preparation of Standard/Sample/Biotin-Antigen compound:

Prepare it within 30 minutes before experiment.

a. When samples and standards are not tested repeatedly:

Take 55µl prepared standard of each gradient and 55µl biotin-labeled antigen working Solution and mix them well in EP tube for later use.

Take 55µl diluted sample and 55ul biotin-labeled antigen working Solution and mix well in EP tube for later use.

b. When the sample and standard are tested in duplicate:

Take 110µl prepared standard of each gradient and 110µl biotin-labeled antigen working Solution and mix them well in EP tube for later use.

Take 110µl diluted sample and 110ul biotin-labeled antigen working Solution and mix well in EP tube for later use.

5. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

Prepare it within 30 minutes before experiment.

a. Calculate required total volume of the working solution: 100µl /well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

b. Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul of SABC into 99 µl of SABC Dilution Buffer.)

Assay Procedure

Washing Notes:

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

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

Procedure:

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

1. Set standard and test samples wells on the pre-coated plate respectively, and then, records their positions. It isrecommended to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard and sample wells!**
2. Add 100µl **Standard/Sample/Biotin-Antigen compound** into each well and incubate for 45 minutes at 37°C. (Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.)
3. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
4. HRP-Streptavidin Conjugate (SABC): Add 100µl SABC Working Solution into each well. Cover it with a new Plate sealer. Incubate for 30 minutes at 37°C.
5. Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
6. TMB Substrate: Add 90µl TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.) (**Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.**)
7. Stop: Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
8. OD Measurement: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

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

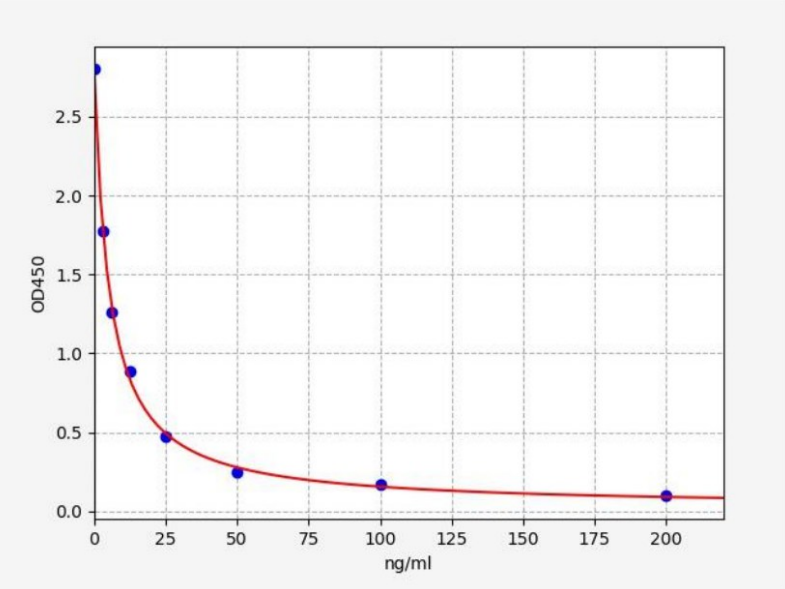
Calculation

Regarding calculation, the standard curve can be plotted as the O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation.

Typical Standard Curve

Results of a typical standard operation of a DPD 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)

STD.(ng/ml)	OD-1	OD-2	Average
0	2.763	2.843	2.803
3.125	1.751	1.801	1.776
6.25	1.239	1.275	1.257
12.5	0.875	0.901	0.888
25	0.468	0.482	0.475
50	0.245	0.253	0.249
100	0.171	0.175	0.173
200	0.097	0.099	0.098



Performance Characteristics

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 month	2-8°C for 6 months
Average (%)	80	95-100

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

Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Range

3.125-200ng/ml

Sensitivity

1.875ng/ml

Specificity

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

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between DPD 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 DPD 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)	88-105%	86-102%	87-100%
EDTA Plasma(n=5)	83-91%	87-101%	84-98%
Heparin Plasma(n=5)	83-100%	83-99%	82-94%

Recovery

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

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	96-101	99
EDTA Plasma(n=5)	86-101	92
Heparin Plasma(n=5)	91-103	99

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. After opening and before using, keep plate dry.

3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don' t let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don' t reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

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