KAMIYA BIOMEDICAL COMPANY

UV-Induced DNA Damage ELISA (CPD Quantitation)

For the rapid detection and quantitation of CPD in any DNA samples

Cat. No. KT-914

For Research Use Only. Not for use in diagnostic procedures.

Product Information

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INTRODUCTION

Absorption of ultraviolet (UV) light produces two predominant types of DNA damage, cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts (6-4PP) (Figure 1). The result is a transition of C to T and CC to TT, which are the most frequent mutations of p53 in both human and mouse skin cancers. UV damaged DNA is usually repaired by nucleotide excision repair (NER) or base excision repair (BER). After UV exposure, cells activate p53 and stall the cell cycle for repair. If the damage is too severe, the cell will trigger apoptosis to get rid of DNA damaged, potentially mutant cells.

UV-induced DNA Damage ELISA Kit (CPD Quantitation) is an enzyme immunoassay developed for rapid detection and quantitation of CPD in any DNA samples. The quantity of CPD in unknown sample is determined by comparing its absorbance with that of a known CPD-DNA calibration curve. Each kit provides sufficient reagents to perform up to 96 assays, including calibration curve and unknown samples.

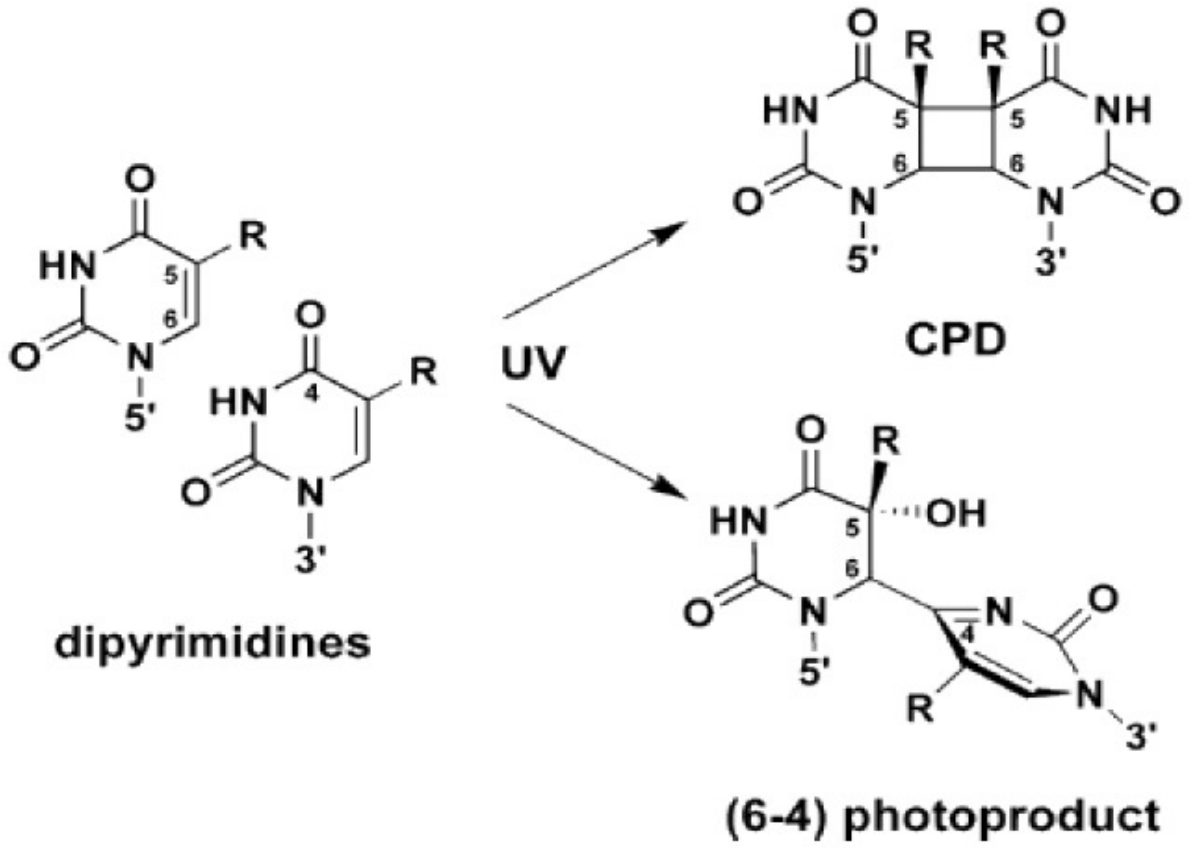

Figure 1: Structures of DNA lesions induced by UV Light

ASSAY PRINCIPLE

CDP-DNA calibrators or unknown DNA samples are first heat denatured before adsorbed onto a 96-well DNA high-binding plate. The CPDs present in the sample or calibrator are probed with an anti-CPD antibody, followed by an HRP conjugated secondary antibody. The CPD content in an unknown sample is determined by comparing with a calibration curve that is prepared from predetermined CPD-DNA calibrators.

COMPONENTS

1. 96-well DNA High-Binding Plate: One strip well 96-well plate, precoated with DNA binding matrix.
2. Anti-CPD Antibody: One 20 µL vial of anti-CPD.
3. Secondary Antibody, HRP Conjugate: One 50 µL vial.
4. Blocking Reagent (100X): One 300 µL tube.
5. Assay Diluent: One 50 mL bottle.
6. 10X Wash Buffer: One 100 mL bottle.
7. Substrate Solution: One 12 mL amber bottle.
8. Stop Solution: One 12 mL bottle.
9. CPD-DNA Calibrator: One 100 µL vial of 25 µg/mL CPD-DNA in 1X TE Buffer.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. DNA samples such as cell or tissue genomic DNA
2. DNA Extraction Kit
3. Heating Block
4. PBS
5. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
6. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
7. Multichannel micropipette reservoir
8. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

STORAGE

Upon receipt, Store the CPD-DNA calibrator at -20ºC and all other components at 4ºC until their expiration dates.

REAGENT PREPARATION

• 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.

• 1X Blocking Reagent: Prepare the appropriate volume for the number of samples being tested. Immediately prior to using, dilute the provided 100X Blocking Reagent 1:100 in 1X PBS. Do not store.

• Anti-CPD Antibody and Secondary Antibody: Immediately before use dilute the Anti-CPD Antibody 1:1,000 and Secondary Antibody 1:1,000 with Assay Diluent. Do not store diluted solutions.

CALIBRATOR PREPARATION

1. Convert CPD-DNA calibrator (25 µg/mL) to single-stranded DNA by incubating the DNA sample at 95ºC for 10 minutes and rapidly chilling on ice for 10 minutes.
Note: Aliquot and store denatured CPD-DNA calibrator at -20ºC. Repeat the above denaturation step every time you prepare the CPD-DNA calibrator.
2. Dilute desired amount of freshly denatured DNA sample 10 fold to 2.5 µg/mL in cold PBS. For example, add 10 µL of the 25 µg/mL CPD-DNA calibrator to 90 uL of cold PBS. Prepare a dilution series of CPD-DNA calibrators in the concentration range of 0 ng/mL – 250 ng/mL by diluting the denatured CPD-DNA Calibrator in cold PBS according to Table 1 below.

Standard Tubes	2.5 µg/mL Denatured CPD-DNA Standard (µL)	Cold PBS (µL)	CPD-DNA (ng/mL)
1	80	720	250
2	400 of Tube #1	400	125
3	400 of Tube #2	400	62.5
4	400 of Tube #3	400	31.3
5	400 of Tube #4	400	15.6
6	400 of Tube #5	400	7.8
7	400 of Tube #6	400	3.9
8	0	400	0

Table 1. Preparation of CPD-DNA Standards

ASSAY PROCEDURE

1. Extract DNA from cell or tissue samples using a commercial DNA Extraction kit or other desired method.
2. Convert DNA sample to single-stranded DNA by incubating the sample at 95ºC for 10 minutes and rapidly chilling on ice for 10 minutes.
3. Dilute denatured DNA sample to 2 µg/mL or less in cold PBS.
4. Add 100 µL of unknown denatured DNA sample or CPD-DNA calibrators to the wells of the DNA High-Binding plate. Incubate at 37ºC for 2 hours or overnight at 4ºC. Each DNA sample including unknown and calibrator should be assayed in duplicate.
5. Remove the DNA solutions and wash twice with PBS. Blot plate on paper towels to remove excess fluid. Add 150 µL of Assay Diluent to each well and block for 1 hour at room temperature.
6. Aspirate the Assay Diluent from the wells and add 100 µL of the diluted anti-CPD antibody to each well and incubate at room temperature for 1 hour on an orbital shaker.
7. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
8. Add 150 µL of prediluted 1X Blocking Reagent to each well (see Preparation of Reagents Section). Incubate the plate for 60 minutes at room temperature on an orbital shaker. Wash microwell strips 3 times according to step 7 above.
9. Add 100 µL of the diluted Secondary Antibody-Enzyme Conjugate to each well and incubate at room temperature for 1 hour on an orbital shaker.
10. Wash microwell strips 3 times according to step 7 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.
12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a standard microplate reader using 450 nm as the primary wave length.

EXAMPLE OF RESULTS

The following figures demonstrate typical Oxidative UV-induced DNA Damage ELISA (CPD Quantitation) results. One should use the data below for reference only. This data should not be used to interpret actual results.

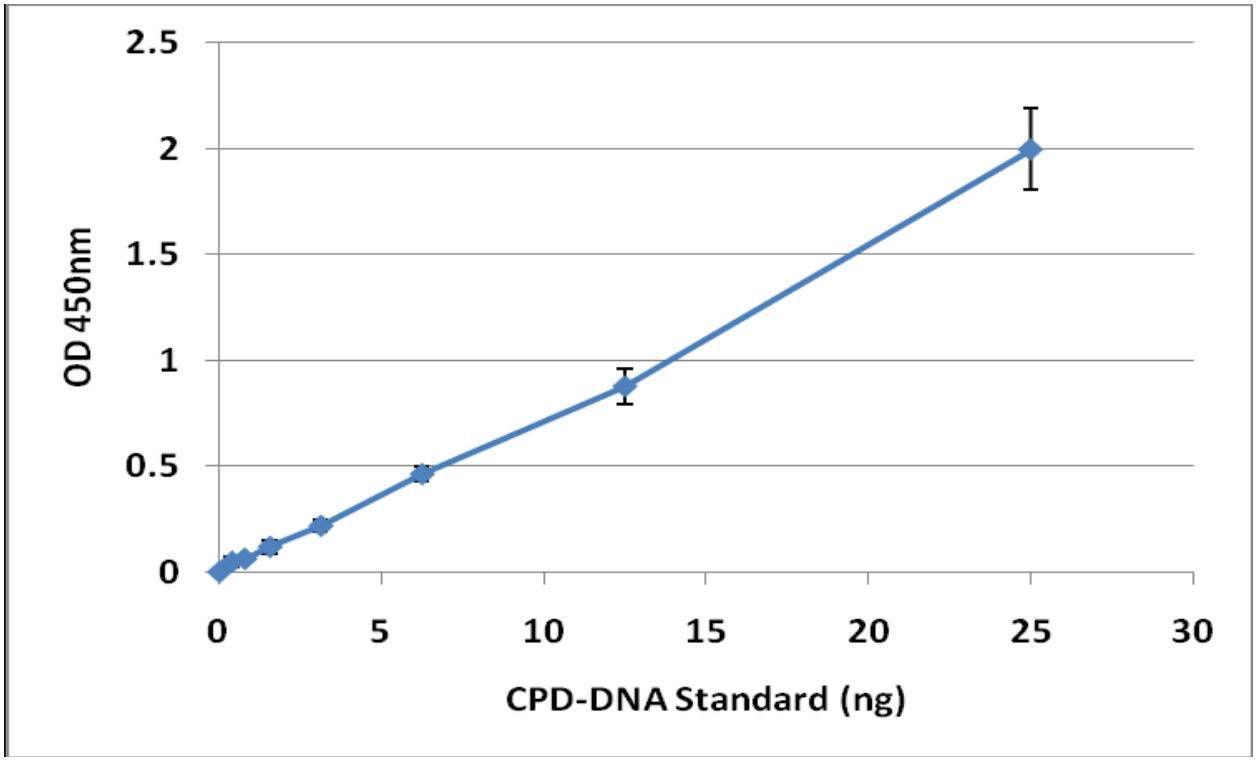

Figure 2: CPD-DNA Calibration Curve.

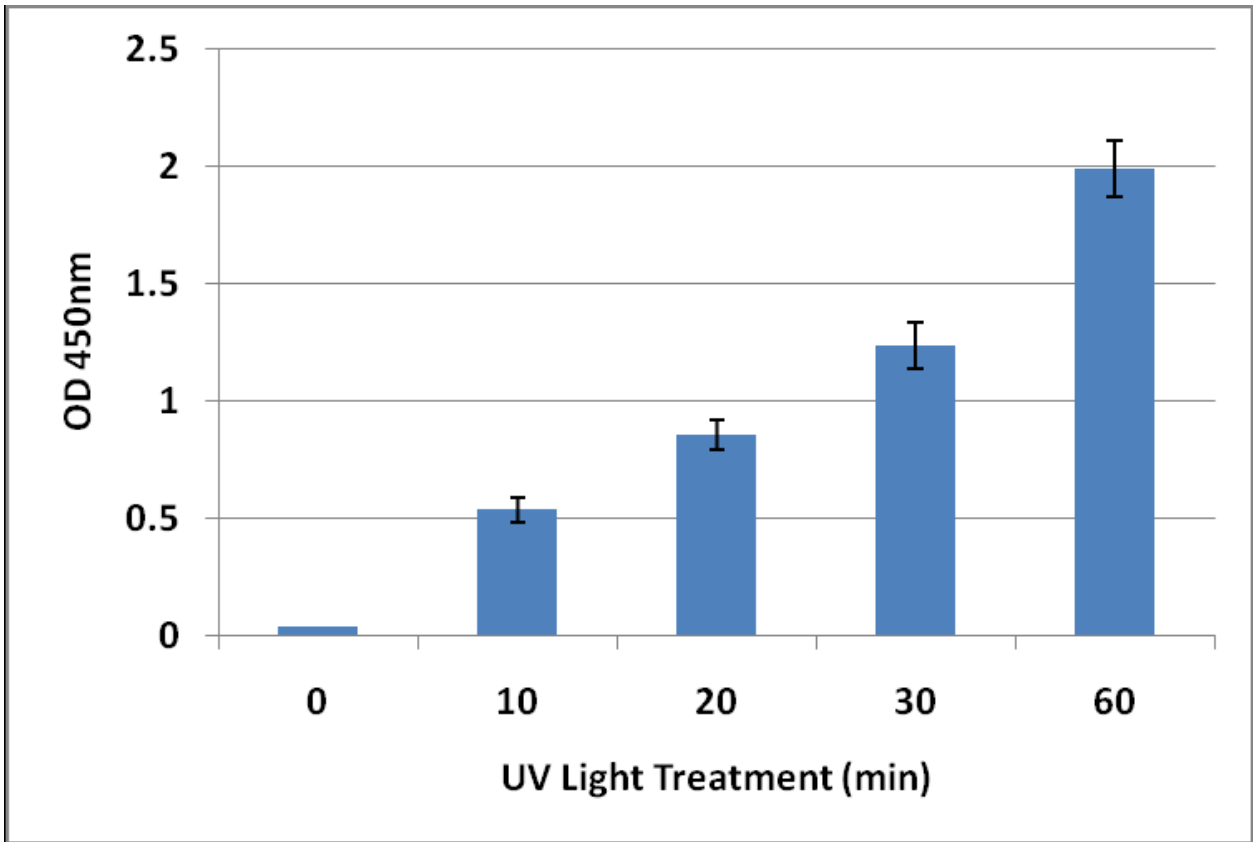

Figure 3: DNA Damage Induced by UV Light. 0.2 mg/mL Calf thymus DNA was exposed to UV light inside a cell culture hood for the time indicated. The CPD levels in 40 ng denatured DNA samples were determined as described in the Assay Protocol.

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