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.

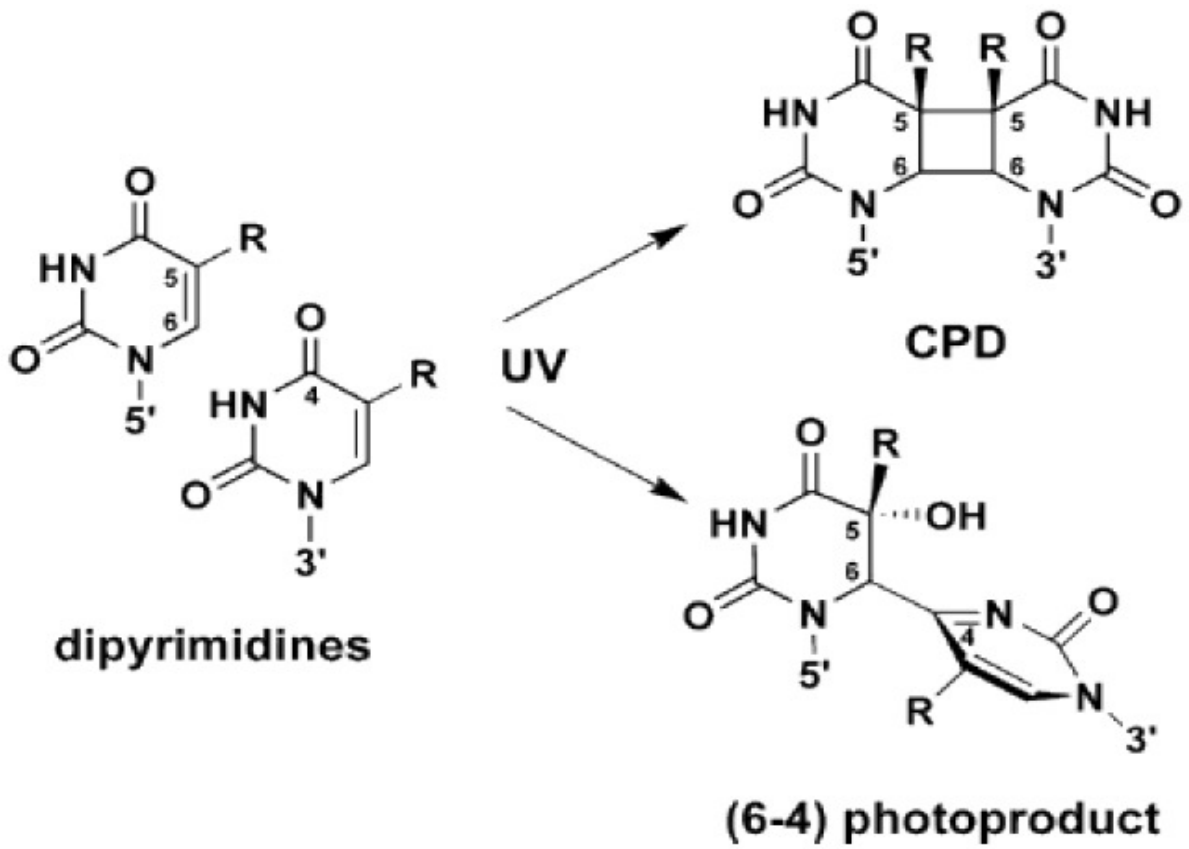

Figure 1: Structures of DNA lesions induced by UV Light

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. DNA High-Binding Plate: One 96-well strip plate.
2. DNA Binding Solution: One 6 mL bottle.
3. Anti-CPD Antibody: One 20 µL vial.
4. Secondary Antibody, HRP Conjugate: One 50 µL vial.
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.
10. Reduced DNA Calibrator: One 100 µL vial of 0.2 mg/mL reduced DNA in 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. 1X TE Buffer (10 mM Tris, pH 8.0, 1 mM EDTA)
6. 10 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
7. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
8. Multichannel micropipette reservoir
9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

STORAGE

Upon receipt, aliquot and store the Reduced DNA and CPD-DNA Calibrators at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

REAGENT PREPARATION

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

• 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) and Reduced DNA (200 µg/mL) to single-stranded DNA by incubating the DNA at 95ºC for 10 minutes and rapidly chilling on ice for 10 minutes.
Note: Aliquot and store denatured DNA at -20ºC. Repeat the above denaturation step every time you prepare the CPD-DNA calibrator.
2. Freshly prepare 4 µg/mL of CPD-DNA by diluting the denatured 25 µg/mL stock in cold TE Buffer.
Example: Add 8 µL to 42 µL of cold TE Buffer.
3. Freshly prepare 4 µg/mL of Reduced DNA by diluting the denatured 200 µg/mL stock in cold TE Buffer.
Example: Add 40 µL to 1.96 mL of cold TE Buffer.
4. Prepare a series of CPD-DNA calibrators according to Table 1.

Standard Tubes	4 µg/mL Denatured CPD-DNA (µL)	4 µg/mL Denatured Reduced DNA (µL)	CPD-DNA Conc. (ng/mL)
1	10	390	100
2	200 of tube #1	200	50
3	200 of tube #2	200	25
4	200 of tube #3	200	12.5
5	200 of tube #4	200	6.25
6	200 of tube #5	200	3.13
7	200 of tube #6	200	1.56
8	0	200	0

Table 1. Preparation of CPD-DNA Calibrators

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 DNA samples to 4 µg/mL in cold TE Buffer.

Note: Samples with high concentrations of CPD may be further diluted 2-4 fold in 4 µg/mL Reduced DNA. A titration may be performed to ensure the samples fall in the range of the calibration curve.

4. Add 50 µL of unknown DNA samples or CPD-DNA calibrators to the wells of the DNA High-Binding plate.
5. Add 50 µL of DNA Binding Solution to each well. Mix well by pipetting and incubate at room temperature overnight on an orbital shaker. Each DNA sample including unknown and calibrator should be assayed in duplicate.
6. Remove the DNA solutions and wash twice with PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hour at room temperature.
7. Remove the Assay Diluent. Blot plate on paper towels to remove excess fluid.
8. Add 100 µL of the diluted Anti-CPD antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker.
9. Wash 5 times with 250 µL of 1X Wash Buffer 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.
10. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 5 times according to step 9 above.
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.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

12. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
13. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length. Use the Reduced DNA Calibrator as an absorbance blank.

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.

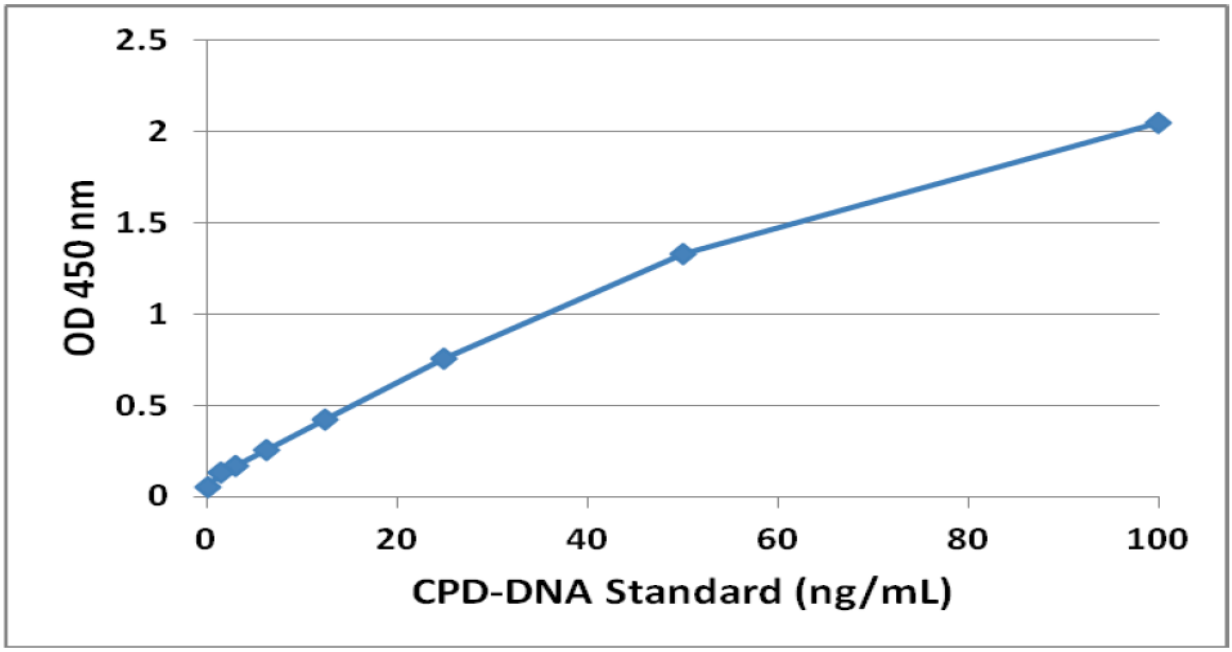

Figure 2: CPD-DNA Calibration Curve.

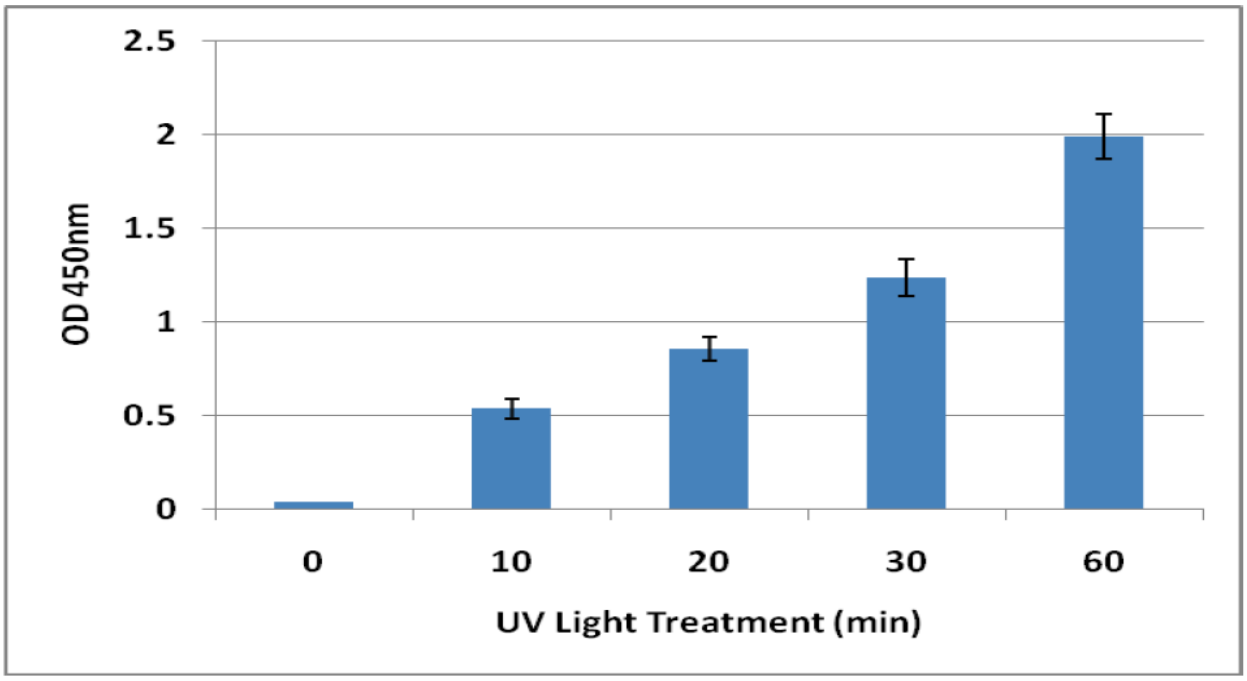

Figure 3: DNA Damage Induced by UV Light. 0.2 mg/mL Calf thymus DNA was exposed to UV light inside a cell culture hood for the time indicated. The CPD levels in denatured DNA samples were determined as described in the Assay Protocol.

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