KAMIYA BIOMEDICAL COMPANY

Cellular UV-Induced DNA Damage ELISA Kit (CPD)

For the rapid detection of CPDs in genomic DNA of cultured cells

Cat. No. KT-917

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.

Cellular UV-induced DNA Damage ELISA Kit (CPD) is an enzyme immunoassay developed for rapid detection of CPDs in genomic DNA of cultured cells. Each kit provides sufficient reagents to perform up to 96 assays.

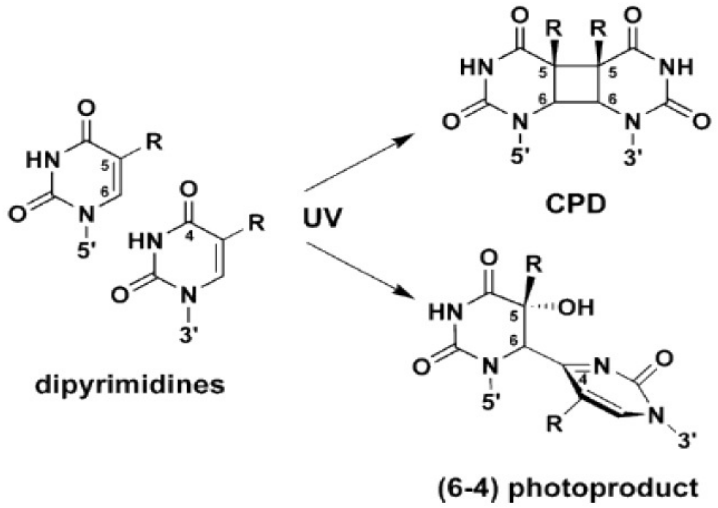

Figure 1: Structures of DNA lesions induced by UV Light

ASSAY PRINCIPLE
Cells are first seeded in a 96-well tissue culture plate. Wells are then UV irradiated to induce DNA damage. After fixation and denaturation, cells containing CPD damage are probed with an anti-CPD antibody, followed by an HRP conjugated secondary antibody. The unbound secondary antibody is removed during a wash step, and substrate solution reactive with HRP is added to the wells. The reaction is terminated by addition of acid and absorbance is measured at 450 nm.

COMPONENTS
1. Anti-CPD Antibody, 100X: One 100 µL vial.
2. Secondary Antibody, HRP Conjugate: One 50 µL amber vial.
3. Denaturation Solution A, 100X: One 200 µL vial.
4. Denaturation Solution B, 100X: One 200 µL vial.
5. Assay Diluent: One 50 mL bottle.
6. 10X Wash Buffer: One 50 mL bottle.
7. Substrate Solution: One 12 mL amber bottle.
8. Stop Solution: One 12 mL bottle.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 96-well tissue culture plate
2. Cell line of interest
3. UV crosslinker, irradiator, or germicidal lamp
4. DPBS containing magnesium and calcium
5. 75% Methanol/25% Acetic Acid
6. 70% Ethanol
7. 10 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
8. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

STORAGE

Store all kit 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, HRP Conjugate: Immediately before use dilute the Anti-CPD Antibody 1:100 and Secondary Antibody 1:1,000 with Assay Diluent. Do not store diluted solutions.
- Denaturation Solution A: Immediately before use dilute the Denaturation Solution A 1:100 with 70% Ethanol. Do not store diluted solution.
- Denaturation Solution B: Immediately before use dilute the Denaturation Solution B 1:100 with DPBS (containing magnesium and calcium). Do not store diluted solution.

ASSAY PROCEDURE

I. Cell Seeding

1. Harvest and resuspend cells in culture medium at 2-4 x 10^5 cells/mL. Seed 100 µL in each well of a 96-well tissue culture plate and incubate overnight at 37ºC, 5% CO_2 (cells should be > 80% confluent).

II. UV Treatment, Fixation and Denaturation

1. Carefully remove medium from the wells by tilting the plate and aspirating from the edge. Gently add 100 µL of DPBS (containing magnesium and calcium) to each well, taking care not to dislodge the cells.

2. Perform UV irradiation to desired wells (removal of plate cover is recommended). Include wells without irradiation as a negative control. Samples should be performed in triplicate.

3. Aspirate the wells and add 100 µL of 75% Methanol/25% Acetic Acid to each well. Incubate 30 minutes at room temperature.

4. Aspirate the wells and add 100 µL of 70% Ethanol to each well. Incubate 30 minutes at room temperature.

5. Aspirate the wells and add 100 µL of Denaturation Solution A (see Preparation of Reagents) to each well. Incubate 5 minutes at room temperature.

6. Gently wash 3 times with 200 µL DPBS (containing magnesium and calcium).

7. Aspirate the wells and add 100 µL of Denaturation Solution B (see Preparation of Reagents) to each well. Incubate 10 minutes at room temperature.

8. Aspirate the wells and add 200 µL of Assay Diluent to each well. Block the wells 30 minutes at room temperature.

III. CPD Detection

1. Aspirate the wells and add 100 µL of the diluted anti-CPD antibody (see Preparation of Reagents) to each well. Incubate at room temperature for 1 hour on an orbital shaker.

2. Wash microwell strips 4 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.

3. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate (see Preparation of Reagents) to each well. Incubate at room temperature for 1 hour on an orbital shaker.

4. Wash microwell strips 4 times according to step 2 above. Proceed immediately to the next step.

5. 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 5-20 minutes.

6. 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).

7. 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 Cellular UV-induced DNA Damage ELISA Kit (CPD) results. One should use the data below for reference only. This data should not be used to interpret actual results.

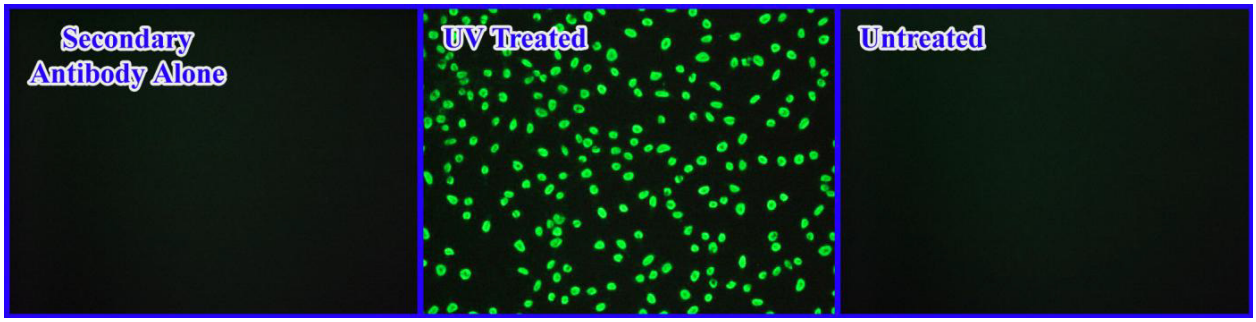

Figure 2: DNA Damage Induced by UV Light in HeLa Cells. HeLa cells were seeded at 20K/well overnight, then exposed to light under a germicidal lamp for 30 minutes. Immunofluorescence staining of CPD damage was determined using Cat. No. KT-918.

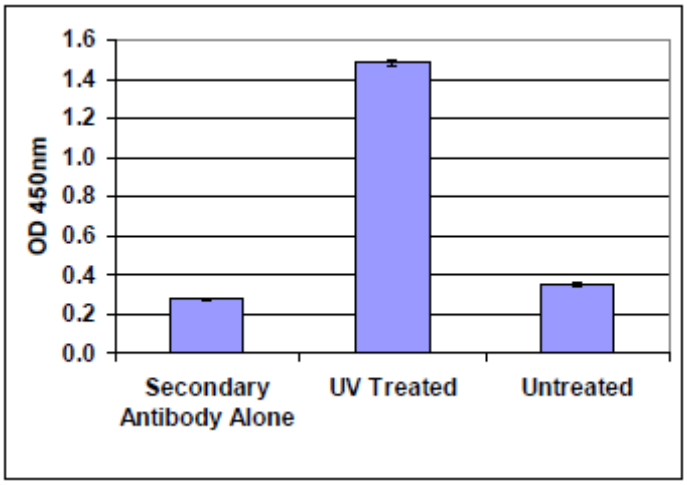

Figure 3: DNA Damage Induced by UV Light in HeLa Cells. HeLa cells were seeded at 20K/well overnight, then exposed to light under a germicidal lamp for 30 minutes. Relative CPD damage was determined as described in the Assay Instructions.

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