Datasheet for ABIN2344980

# OxiSelect™ Cellular UV-Induced DNA Damage Staining Kit (CPD)

2 Images | 2 Publications

Go to Product page

## Overview

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| Quantity: | 96 tests |
| Reactivity: | Others |
| Application: | Cellular Assay (CA) |

## Product Details

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| Brand: | OxiSelect™ |
| Sample Type: | Cell Samples |
| Detection Method: | Fluorometric |
| Characteristics: | OxiSelect™ Cellular UV-induced DNA Damage Staining Kit (CPD) is an immunofluorescence assay developed for rapid detection of CPDs in genomic DNA of cultured cells. Each kit provides sufficient reagents for up to 96 stainings in a 96-well plate. Figure 1: Structures of DNA lesions induced by UV Light |
| Components: | 1. Anti-CPD Antibody, 100X : One 100 µL vial.<br>2. Secondary Antibody, FITC Conjugate, 100X : One 100 µL amber vial.<br>3. Denaturation Solution A, 100X : One 200 µL vial.<br>4. Denaturation Solution B, 100X : One 200 µL vial.<br>5. Assay Diluent : One 50 mL bottle.<br>6. 10X Wash Buffer : One 50 mL bottle. |
| Material not included: | 1. 96-well tissue culture plate<br>2. Cell line of interest<br>3. UV crosslinker, irradiator, or germicidal lamp<br>4. DPBS containing magnesium and calcium<br>5. 75 % Methanol/25 % Acetic Acid |

## Product Details

6. 70 % Ethanol
7. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
8. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
9. Fluorescence microscope with FITC filter

## Target Details

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| Background: | 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. |

## Application Details

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| Application Notes: | Optimal working dilution should be determined by the investigator. |
| Comment: | • Measure CPD structures of DNA lesions induced by UV light<br>• Detect structures by immunofluorescence staining under a fluorescence microscope |
| Protocol: | 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 a FITC conjugated secondary antibody. The unbound secondary antibody is removed during a wash step, and stained cells can then be visualized with a fluorescence microscope. |
| 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, FITC Conjugate: Immediately before use dilute the Anti-CPD Antibody 1:100 and Secondary Antibody 1:100 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: | The following assay protocol is written for a 96-well format. Refer to the below table for the appropriate dispensing volumes of other plate formats. Note: using other plate formats will decrease the number of assays possible with this kit. Culture Dish 96-well 48-well 24-well 12- |

well 6-well DPBS during UV 100 200 400 800 1600 Irradiation (µL/well) 75 % Methanol/ 25 % Acetic Acid 100 200 400 800 1600 (µL/well) 70 % Ethanol (µL/well) 100 200 400 800 1600 Denaturation Solution A 100 200 400 800 1600 (µL/well) DPBS during Washing 200 400 800 1600 3200 (µL/well) Denaturation Solution B 100 200 400 800 1600 (µL/well) Assay Diluent Blocking 200 400 800 1600 3200 (µL/well) 1X Anti-CPD Antibody 100 200 400 800 1600 Solution (µL/well) 1X Secondary Antibody, FITC Conjugate Solution 100 200 400 800 1600 (µ L/well) Wash Buffer (µL/well) 250 500 1000 2000 4000 Table

1. Dispensing Volumes of Different Plate Formats. 4

I. Cell Seeding 5

1. Harvest and resuspend cells in culture medium at 2-4 x 10 cells/mL. Seed 100 µL in each well of a 96-well tissue culture plate and incubate overnight at 37 °C, 5 % CO2 (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 Immunofluorescence Staining

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, FITC 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. Add 100 L of DPBS to each well.
6. View staining with a fluorescence microscope using FITC filter. 5

## Application Details

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| Restrictions: | For Research Use only |

## Handling

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| Storage: | 4 °C |
| Storage Comment: | Store all kit components at 4°C until their expiration dates. |

## Publications

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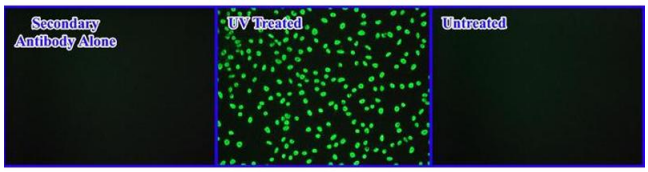
| Product cited in: | Hasegawa, Nakashima, Suzuki: "Nuclear DNA damage-triggered NLRP3 inflammasome activation promotes UVB-induced inflammatory responses in human keratinocytes." in: **Biochemical and biophysical research communications**, Vol. 477, Issue 3, pp. 329-35, (2016) ( PubMed).<br><br>Núñez-Lozano, Pimentel, Castro-Smirnov, Calvo, Míguez, de la Cueva-Méndez: "Biocompatible films with tailored spectral response for prevention of DNA damage in skin cells." in: **Advanced healthcare materials**, Vol. 4, Issue 13, pp. 1944-8, (2015) (PubMed). |

## Images




**Immunofluorescence**

**Image 1.** 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 as described in the Assay Protocol for #STA-327.

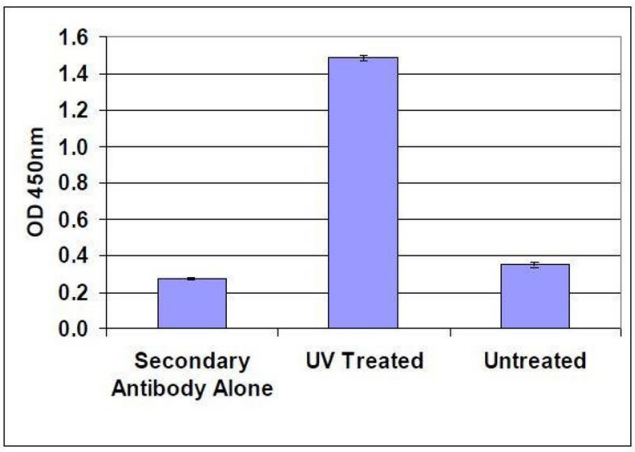



**Image 2.** DNA Damage Induced by UV Light in HeLa Cells. HeLa cells were seeded at 20K/well overnight, then exposed to light under a germicidal lamp for 30 minutes. Relative CPD damage was determined using the OxiSelect™ Cellular UV-induced DNA Damage ELISA Kit