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Product Manual

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

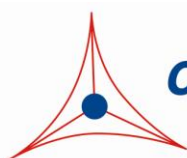
Catalog Number

STA-327-T

32 assays

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

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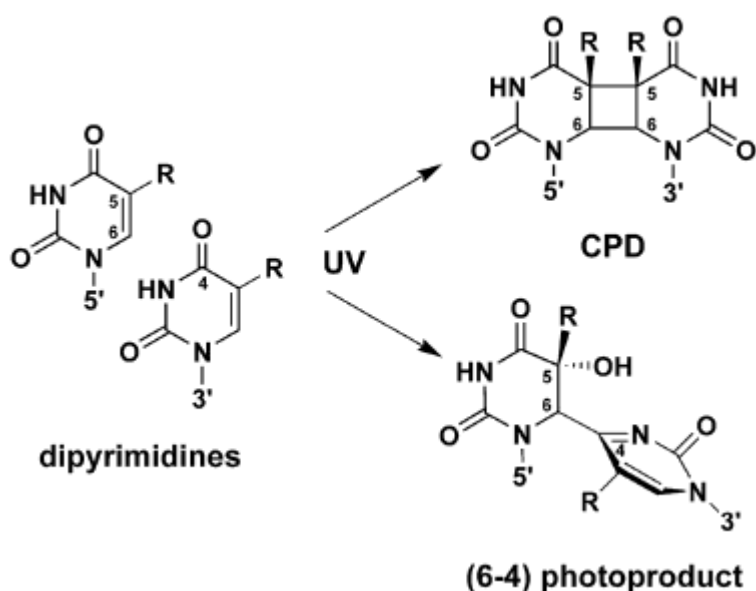


**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **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.

Cell Biolabs' 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. This Trial Size kit provides sufficient reagents for up to 32 stainings in a 96-well plate.



**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 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.

## **Related Products**

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-321: OxiSelect™ DNA Double-Strand Break (DSB) Staining Kit
3. STA-322: OxiSelect™ UV-induced DNA Damage ELISA Kit (CPD Quantitation)
4. STA-323: OxiSelect™ UV-induced DNA Damage ELISA Kit (6-4PP Quantitation)
5. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites)
6. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
7. STA-326: OxiSelect™ Cellular UV-induced DNA Damage ELISA Kit (CPD)
8. STA-328: OxiSelect™ Cellular UV-induced DNA Damage ELISA Kit (6-4PP)
9. STA-329: OxiSelect™ Cellular UV-induced DNA Damage Staining Kit (6-4PP)
10. STA-351: OxiSelect™ Comet Assay Kit (3-Well Slides), 75 Assays
11. STA-353: OxiSelect™ Comet Assay Slides (3-Well), 25 Slides
12. STA-355: OxiSelect™ 96-Well Comet Assay Kit

## **Kit Components**

1. Anti-CPD Antibody, 100X (Part No. 232601-T): One 40 µL vial.
2. Secondary Antibody, FITC Conjugate, 100X (Part No. 232102-T): One 40 µL amber vial.
3. Denaturation Solution A, 100X (Part No. 232602-T): One 75 µL vial.
4. Denaturation Solution B, 100X (Part No. 232603-T): One 75 µL vial.
5. Assay Diluent (Part No. 310804-T): One 20 mL bottle.
6. 10X Wash Buffer (Part No. 310806-T): One 30 mL bottle.

## **Materials 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 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

## **Storage**

Store all kit components at 4°C until their expiration dates.

## **Preparation of Reagents**

- 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 Protocol**

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.*

<b>Culture Dish</b>	<b>96-well</b>	<b>48-well</b>	<b>24-well</b>	<b>12-well</b>	<b>6-well</b>
DPBS during UV Irradiation (μL/well)	100	200	400	800	1600
75% Methanol/ 25% Acetic Acid (μL/well)	100	200	400	800	1600
70% Ethanol (μL/well)	100	200	400	800	1600
Denaturation Solution A (μL/well)	100	200	400	800	1600
DPBS during Washing (μL/well)	200	400	800	1600	3200
Denaturation Solution B (μL/well)	100	200	400	800	1600
Assay Diluent Blocking (μL/well)	200	400	800	1600	3200
1X Anti-CPD Antibody Solution (μL/well)	100	200	400	800	1600
1X Secondary Antibody, FITC Conjugate Solution (μL/well)	100	200	400	800	1600
Wash Buffer (μL/well)	250	500	1000	2000	4000

**Table 1. Dispensing Volumes of Different Plate Formats.**

## I. Cell Seeding

1. Harvest and resuspend cells in culture medium at  $2-4 \times 10^5$  cells/mL. Seed 100  $\mu$ L in each well of a 96-well tissue culture plate and incubate overnight at 37°C, 5% CO<sub>2</sub> (cells should be > 80% confluent).

## II. UV Treatment, Fixation and Denaturation

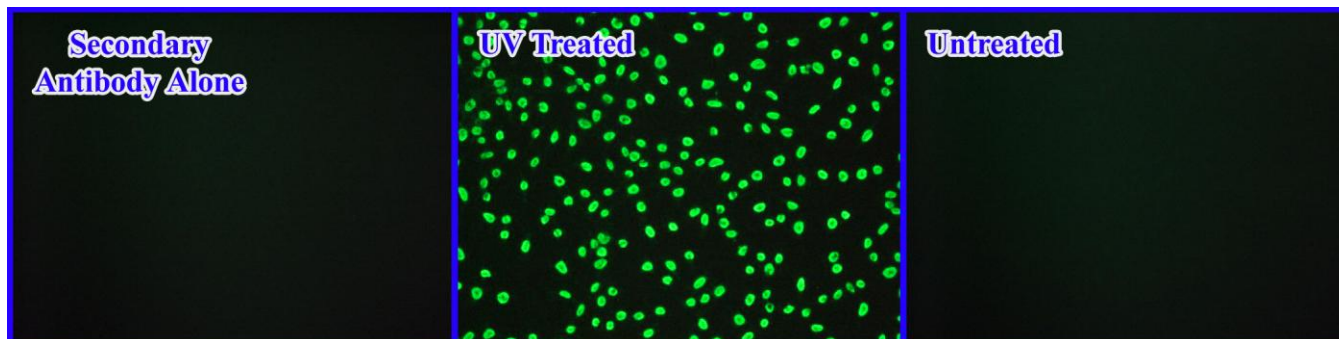
1. Carefully remove medium from the wells by tilting the plate and aspirating from the edge. Gently add 100  $\mu$ L of DPBS (containing magnesium and calcium) to each well, taking care not to dislodge the cells.
2. Perform UV irradiation to desired wells (removal of plate cover is recommended). Include wells without irradiation as a negative control. Samples should be performed in triplicate.
3. Aspirate the wells and add 100  $\mu$ L of 75% Methanol/25% Acetic Acid to each well. Incubate 30 minutes at room temperature.
4. Aspirate the wells and add 100  $\mu$ L of 70% Ethanol to each well. Incubate 30 minutes at room temperature.
5. Aspirate the wells and add 100  $\mu$ L of Denaturation Solution A (see Preparation of Reagents) to each well. Incubate 5 minutes at room temperature.
6. Gently wash 3 times with 200  $\mu$ L DPBS (containing magnesium and calcium).
7. Aspirate the wells and add 100  $\mu$ L of Denaturation Solution B (see Preparation of Reagents) to each well. Incubate 10 minutes at room temperature.
8. Aspirate the wells and add 200  $\mu$ L of Assay Diluent to each well. Block the wells 30 minutes at room temperature.

## III. CPD Immunofluorescence Staining

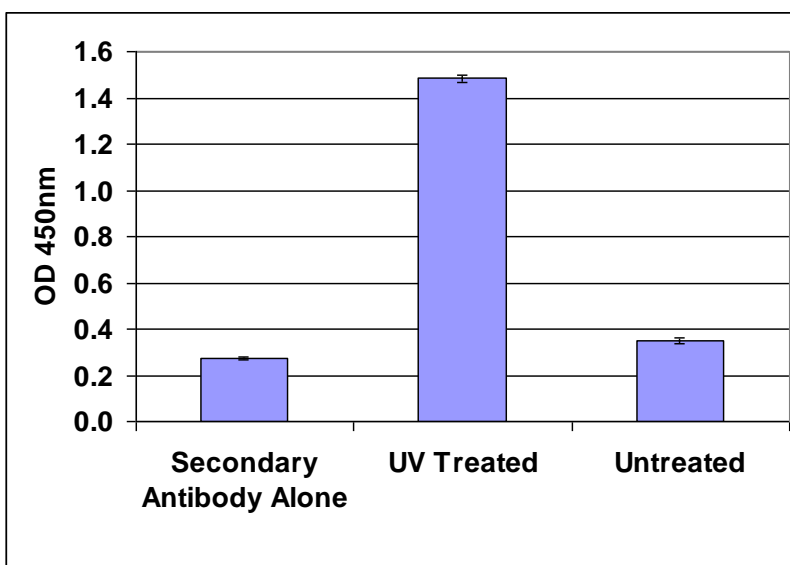
1. Aspirate the wells and add 100  $\mu$ L of the diluted anti-CPD antibody (see Preparation of Reagents) to each well. Incubate at room temperature for 1 hour on an orbital shaker.
2. Wash microwell strips 4 times with 250  $\mu$ L 1X Wash Buffer per well with thorough aspiration between each wash. 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  $\mu$ L 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  $\mu$ L of DPBS to each well.
6. View staining with a fluorescence microscope using FITC filter.

## Example of Results

The following figures demonstrate typical Cellular UV-induced DNA Damage Staining 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 as described in the Assay Instructions.



**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 using the OxiSelect™ Cellular UV-induced DNA Damage ELISA Kit (STA-326).

## **References**

1. Lippke JA, Gordon LK, Brash DE, Haseltine WA. (1981) *Proc Natl Acad Sci U S A*. **78**:3388–3392.
2. Mitchell DL, Nairn RS. (1989) *Photochem Photobiol*. **49**:805–819.
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7. Hill LL, Ouhtit A, Loughlin SM, Kripke ML, Ananthaswamy HN, Owen-Schaub LB. (1999) *Science*. **285**:898–900.

## **Recent Product Citations**

1. Hasegawa, T. et al. (2016). Nuclear DNA damage-triggered NLRP3 inflammasome activation promotes UVB-induced inflammatory responses in human keratinocytes. *Biochem Biophys Res Commun*. doi:10.1016/j.bbrc.2016.06.106.
2. Núñez-Lozano, R. et al. (2015). Biocompatible films with tailored spectral response for prevention of DNA damage in skin cells. *Adv Healthc Mater*. doi: 10.1002/adhm.201500223.

## **Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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