

---

Product Manual

# OxiSelect™ Cellular UV-Induced DNA Damage ELISA Kit (6-4PP)

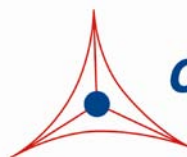
Catalog Number

STA-328

96 assays

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

---

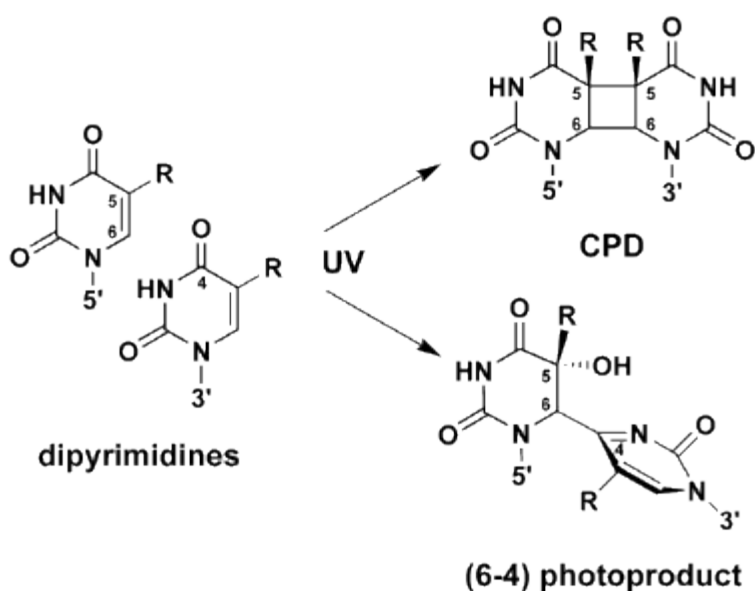


**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **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 ELISA Kit (6-4PP) is an enzyme immunoassay developed for rapid detection of 6-4PPs 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 6-4PP damage are probed with an anti-6-4PP 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.

## **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-327: OxiSelect™ Cellular UV-induced DNA Damage Staining Kit (CPD)
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-6-4PP Antibody, 100X (Part No. 232801): One 100 µL vial.
2. Secondary Antibody, HRP Conjugate (Part No. 10902): One 50 µL vial.
3. Denaturation Solution A, 100X (Part No. 232602): One 200 µL vial.
4. Denaturation Solution B, 100X (Part No. 232603): One 200 µL vial.
5. Assay Diluent (Part No. 310804): One 50 mL bottle.
6. 10X Wash Buffer (Part No. 250007): One 50 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 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. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

## **Storage**

Store all kit components at 4°C.

## **Preparation of Reagents**

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-6-4PP Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute the Anti-6-4PP Antibody 1:100 and Secondary Antibody 1:1000 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**

### **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**

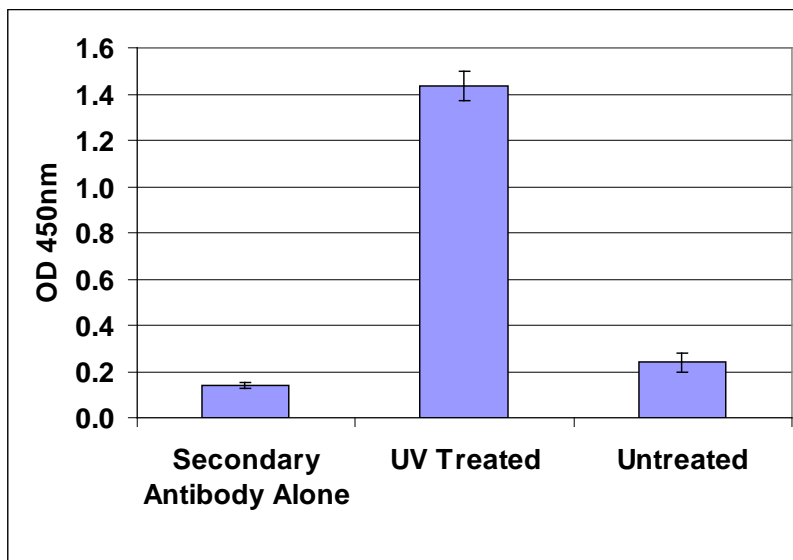
1. Carefully remove medium from the wells by tilting the plate and aspirating from the edge. Gently add 100  $\mu$ L of DPBS (containing magnesium and calcium) to each well, taking care not to dislodge the cells.
2. Perform UV irradiation to desired wells (removal of plate cover is recommended). Include 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. 6-4PP Detection

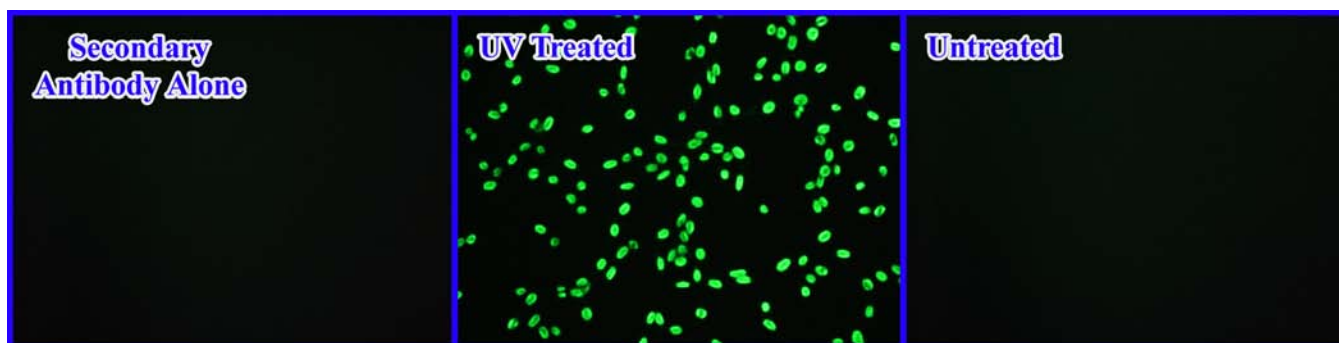
1. Aspirate the wells and add 100  $\mu$ L of the diluted anti-6-4PP antibody (see Preparation of Reagents) to each well. Incubate at room temperature for 1 hour on an orbital shaker.
2. Wash microwell strips 4 times with 250  $\mu$ L 1X Wash Buffer per well with thorough aspiration between each wash. 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, 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  $\mu$ L 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  $\mu$ L 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 (6-4PP) 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 NIH3T3 Cells.** NIH3T3 cells were seeded at 20K/well overnight, then exposed to light under a germicidal lamp for 30 minutes. Relative 6-4PP damage was determined as described in the Assay Instructions.



**Figure 3: DNA Damage Induced by UV Light in NIH3T3 Cells.** NIH3T3 cells were seeded at 20K/well overnight, then exposed to light under a germicidal lamp for 30 minutes. Immuno-fluorescence staining of 6-4PP damage was determined using the OxiSelect™ Cellular UV-induced DNA Damage Staining Kit (STA-329).

## **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.
3. Ananthaswamy HN, Loughlin SM, Cox P, Evans RL, Ullrich SE, Kripke ML. (1997) *Nat Med*. **3**:510–514.
4. Soehnge H, Ouhtit A, Ananthaswamy ON. (1997) *Front Biosci*. **2**:D538–D551.
5. el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. (1993) *Cell*. **75**:817–825.
6. Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, Thiagalingam S, Kinzler KW, Vogelstein B. (1997) *Mol Cell*. **1**:3–11.
7. Hill LL, Ouhtit A, Loughlin SM, Kripke ML, Ananthaswamy HN, Owen-Schaub LB. (1999) *Science*. **285**:898–900.

## **Recent Product Citation**

Kuschal, C. et al. (2013). Repair of UV photolesions in xeroderma pigmentosum group C cells induced by translational readthrough of premature termination codons. *PNAS*. **110**:19483-19488.

## **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.

## **Contact Information**

Cell Biolabs, Inc.  
7758 Arjons Drive  
San Diego, CA 92126  
Worldwide: +1 858-271-6500  
USA Toll-Free: 1-888-CBL-0505  
E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

©2013-2016: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.