
Product Manual

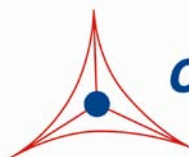
OxiSelect™ Cellular UV-Induced DNA Damage Staining Kit (6-4PP)

Catalog Number

STA-329

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 Staining Kit (6-4PP) is an immunofluorescence assay developed for rapid detection of 6-4PPs in genomic DNA of cultured cells. Each kit provides sufficient reagents for up to 96 stainings in a 96-well plate.

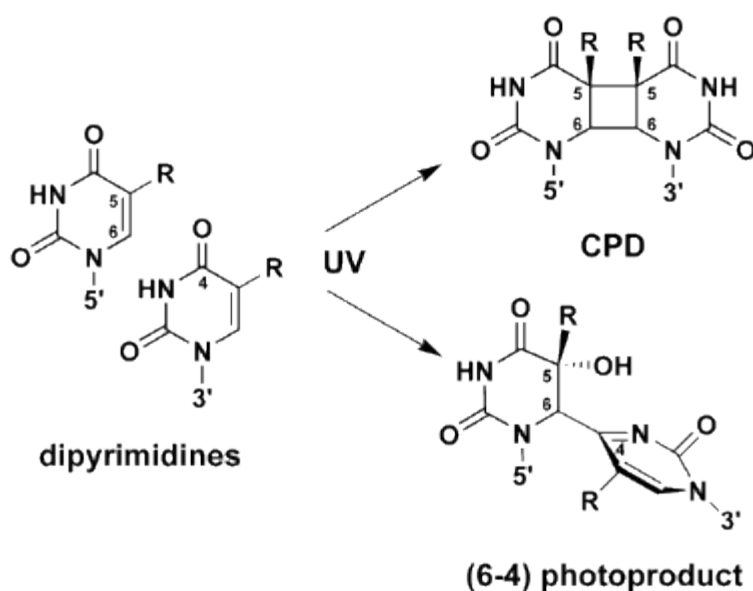


Figure 1: Structures of DNA lesions induced by UV Light

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 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-327: OxiSelect™ Cellular UV-induced DNA Damage Staining Kit (CPD)
9. STA-328: OxiSelect™ Cellular UV-induced DNA Damage ELISA 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, FITC Conjugate, 100X (Part No. 232102): One 100 µL amber 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.

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-6-4PP Antibody and Secondary Antibody, FITC Conjugate: Immediately before use dilute the Anti-6-4PP 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.

| Culture Dish | 96-well | 48-well | 24-well | 12-well | 6-well |
|--|----------------|----------------|----------------|----------------|---------------|
| 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-6-4PP 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 μ L 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 μ 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 μ L of 75% Methanol/25% Acetic Acid to each well. Incubate 30 minutes at room temperature.
4. Aspirate the wells and add 100 μ L of 70% Ethanol to each well. Incubate 30 minutes at room temperature.
5. Aspirate the wells and add 100 μ 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 μ L DPBS (containing magnesium and calcium).
7. Aspirate the wells and add 100 μ 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 μ L of Assay Diluent to each well. Block the wells 30 minutes at room temperature.

III. 6-4PP Immunofluorescence Staining

1. Aspirate the wells and add 100 μ 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 μ 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 μ 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 μ 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 (6-4PP) results. One should use the data below for reference only. This data should not be used to interpret actual results.

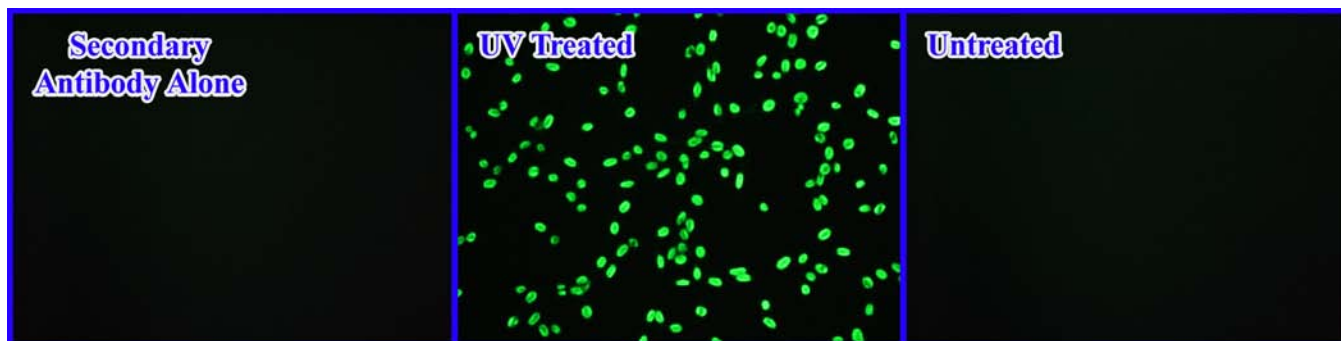


Figure 2: DNA Damage Induced by UV Light in NIH3T3 Cells. NIH3T3 cells were seeded at 20K/well overnight, then exposed to light under a germicidal lamp for 30 minutes. Immuno-fluorescence staining of 6-4PP damage was determined as described in the Assay Instructions.

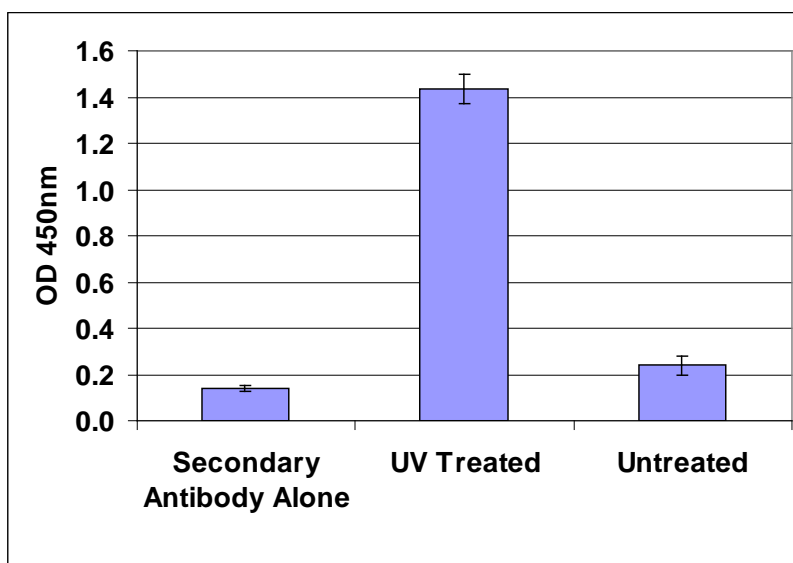


Figure 3: DNA Damage Induced by UV Light in NIH3T3 Cells. NIH3T3 cells were seeded at 20K/well overnight, then exposed to light under a germicidal lamp for 30 minutes. Relative 6-4PP damage was determined using the OxiSelect™ Cellular UV-induced DNA Damage ELISA Kit (STA-328).

References

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Warranty

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