

**NOTE: Revision to
Format and Assay Protocol**

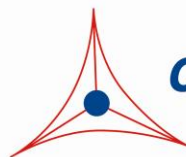
Product Manual

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

Catalog Number

STA-323	96 assays
STA-323-5	5 x 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™ Oxidative UV-induced DNA Damage ELISA Kit (6-4PP Quantitation) is an enzyme immunoassay developed for rapid detection and quantitation of 6-4PP in any DNA samples. The quantity of 6-4PP in unknown sample is determined by comparing its absorbance with that of a known 6-4PP-DNA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

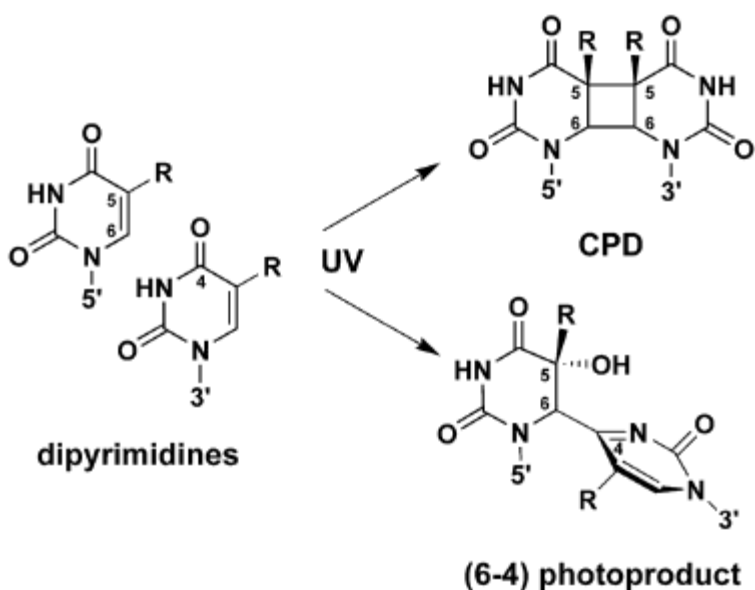


Figure 1: Structures of DNA lesions induced by UV Light

Assay Principle

6-4PP-DNA standards or unknown DNA samples are first heat denatured before being adsorbed onto a 96-well DNA high-binding plate. The 6-4PPs present in the sample or standard are probed with an anti-6-4PP antibody, followed by an HRP conjugated secondary antibody. The 6-4PP content in an unknown sample is determined by comparing with a standard curve that is prepared from predetermined 6-4PP-DNA standards.

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-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites)
5. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
6. STA-326: OxiSelect™ Cellular UV-induced DNA Damage ELISA Kit (CPD)
7. STA-327: OxiSelect™ Cellular UV-induced DNA Damage Staining 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)

Kit Components

1. DNA High-Binding Plate (Part No. 232404): One 96-well strip plate.
2. DNA Binding Solution (Part No. 232405): One 6 mL bottle.
3. Anti-6-4PP Antibody (Part No. 232301): One 20 µL vial.
4. Secondary Antibody, HRP Conjugate (Part No. 10902): One 50 µL vial.
5. Assay Diluent (Part No. 310804): One 50 mL bottle.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part No. 310808): One 12 mL bottle.
9. 6-4PP-DNA Standard (Part No. 232302): One 100 µL vial of 25 µg/mL 6-4PP-DNA in 1X TE Buffer.
10. Reduced DNA Standard (Part No. 232207): One 100 µL vial of 0.2 mg/mL reduced DNA in TE Buffer.

Materials Not Supplied

1. DNA samples such as cell or tissue genomic DNA
2. DNA Extraction Kit
3. Heating Block
4. PBS
5. 1X TE Buffer (10 mM Tris, pH 8.0, 1 mM EDTA)
6. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
7. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
8. Multichannel micropipette reservoir

9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Reduced DNA and 6-4PP-DNA Standards at -20°C to avoid multiple freeze/thaw cycles. Store all other 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: Immediately before use dilute the Anti-6-4PP Antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Convert 6-4PP-DNA standard (25 µg/mL) and Reduced DNA (200 µg/mL) to single-stranded DNA by incubating the DNA at 95°C for 10 minutes and rapidly chilling on ice for 10 minutes.

Note: Aliquot and store denatured DNA at -20°C. Repeat the above denaturation step every time you prepare the 6-4PP-DNA standard.

2. Freshly prepare 4 µg/mL of 6-4PP-DNA by diluting the denatured 25 µg/mL stock in cold TE Buffer. Example: Add 8 µL to 42 µL of cold TE Buffer.
3. Freshly prepare 4 µg/mL of Reduced DNA by diluting the denatured 200 µg/mL stock in cold TE Buffer. Example: Add 40 µL to 1.96 mL of cold TE Buffer.
4. Prepare a series of 6-4PP-DNA standards according to Table 1.

Standard Tubes	4 µg/mL Denatured 6-4PP-DNA (µL)	4 µg/mL Denatured Reduced DNA (µL)	6-4PP-DNA Conc. (ng/mL)
1	10	390	100
2	200 of tube #1	200	50
3	200 of tube #2	200	25
4	200 of tube #3	200	12.5
5	200 of tube #4	200	6.25
6	200 of tube #5	200	3.13
7	200 of tube #6	200	1.56
8	0	200	0

Table 1. Preparation of 6-4PP-DNA Standards

Assay Protocol

1. Extract DNA from cell or tissue samples using a commercial DNA Extraction kit or other desired method.
2. Convert DNA sample to single-stranded DNA by incubating the sample at 95°C for 10 minutes and rapidly chilling on ice for 10 minutes.
3. Dilute DNA samples to 4 µg/mL in cold TE Buffer.
Note: Samples with high concentrations of 6-4PP may be further diluted 2-4 fold in 4 µg/mL Reduced DNA. A titration may be performed to ensure the samples fall in the range of the standard curve.
4. Add 50 µL of unknown DNA samples or 6-4PP-DNA standards to the wells of the DNA High-Binding plate.
5. Add 50 µL of DNA Binding Solution to each well. Mix well by pipetting and incubate at room temperature overnight on an orbital shaker. Each DNA sample including unknown and standard should be assayed in duplicate.
6. Remove the DNA solutions and wash twice with PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hour at room temperature.
7. Remove the Assay Diluent. Blot plate on paper towels to remove excess fluid.
8. Add 100 µL of the diluted Anti-6-4PP Antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker.
9. Wash 5 times with 250 µL of 1X Wash Buffer 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.
10. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 5 times according to step 9 above.
11. 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 2-30 minutes.
Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
13. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length. Use the Reduced DNA Standard as an absorbance blank.

Example of Results

The following figures demonstrate typical Oxidative UV-induced DNA Damage ELISA (6-4PP Quantitation) results. One should use the data below for reference only. This data should not be used to interpret actual results.

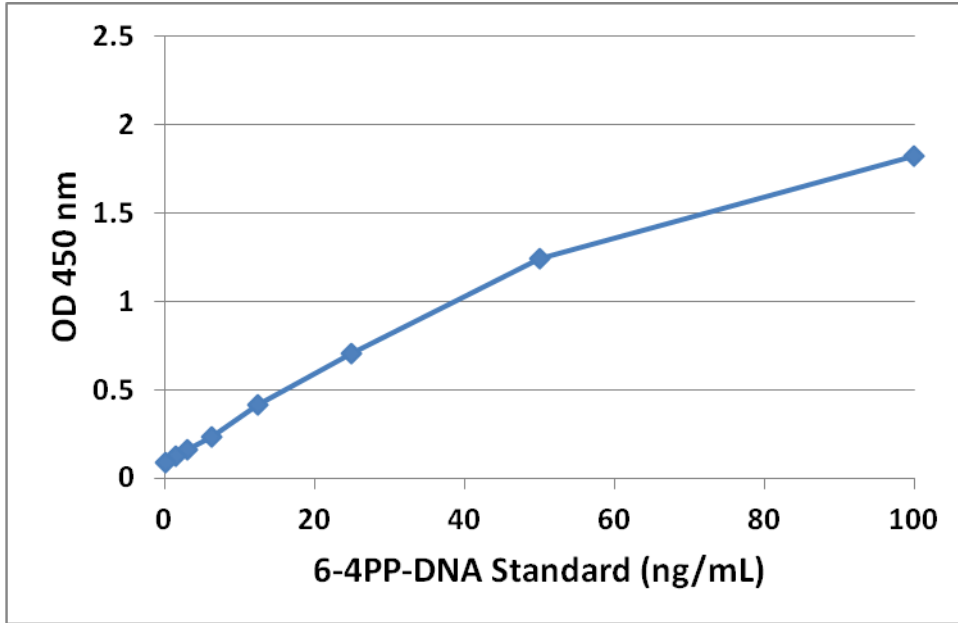


Figure 2: 6-4PP-DNA Standard Curve.

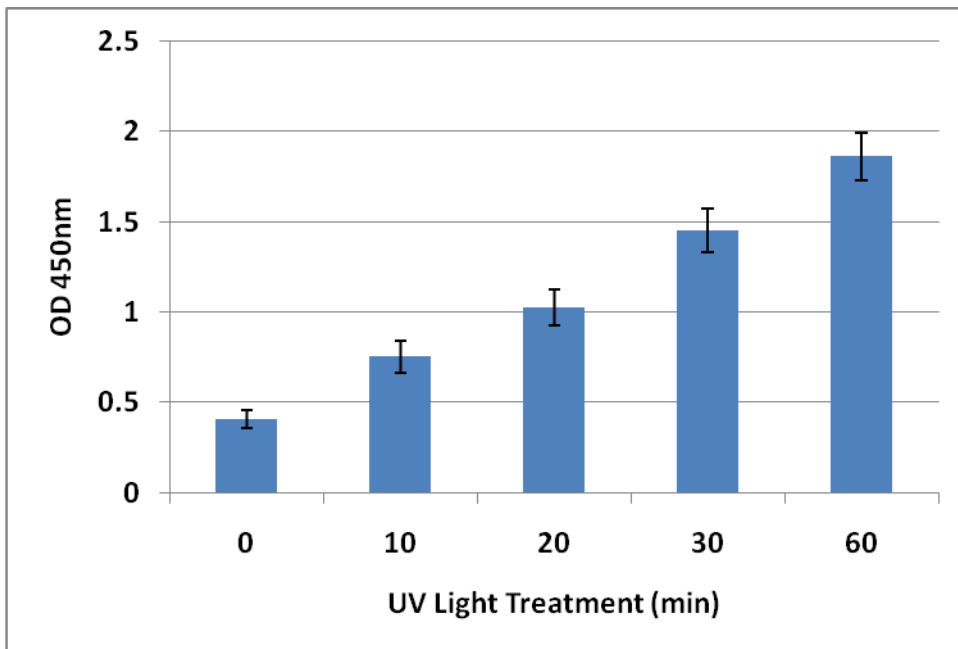


Figure 3: DNA Damage Induced by UV Light. 0.2 mg/mL Calf thymus DNA was exposed to UV light inside a cell culture hood for the time indicated. The 6-4PP levels in denatured DNA samples were determined as described in the Assay Protocol.

References

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Recent Product Citation

Akaike, Y. et al. (2014). Homeodomain-interacting protein kinase 2 regulates DNA damage response through interacting with heterochromatin protein 1 γ . *Oncogene*. doi:10.1038/onc.2014.278.

Warranty

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