Datasheet for ABIN2344965

UV-Induced DNA Damage ELISA Kit

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Overview

Quantity:	96 tests
Target:	UV-Induced DNA Damage
Reactivity:	Others
Method Type:	DNA-Binding ELISA
Application:	ELISA

Product Details

Brand:	OxiSelect™
Sample Type:	DNA samples
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	OxiSelect™ Oxidative UV-induced DNA Damage ELISA Kit (CPD Quantitation) is an enzyme immunoassay developed for rapid detection and quantitation of CPD in any DNA samples. The quantity of CPD in unknown sample is determined by comparing its absorbance with that of a known CPD-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
Components:	1. DNA High-Binding Plate : One 96-well strip plate. 2. DNA Binding Solution : One 6 mL bottle. 3. Anti-CPD Antibody : One 20 µL vial. 4. Secondary Antibody, HRP Conjugate : One 50 µL vial. 5. Assay Diluent : One 50 mL bottle.

Product Details

	6. 10X Wash Buffer : One 100 mL bottle. 7. Substrate Solution : One 12 mL amber bottle. 8. Stop Solution (Part. No. 310808): One 12 mL bottle. 9. CPD-DNA Standard : One 100 µL vial of 25 µg/mL CPD-DNA in 1X TE Buffer. 10. Reduced DNA Standard : One 100 µL vial of 0.2 mg/mL reduced DNA in TE Buffer.
Material not included:	1. DNA samples such as cell or tissue genomic DNA 2. DNA Extraction Kit 3. Heating Block 4. PBS 5. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 6. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 7. Multichannel micropipette reservoir 8. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Target Details

Target:	UV-Induced DNA Damage
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

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Measure CPD structures of DNA lesions induced by UV light • Detect structures in isolated DNA using standard ELISA plate format
Plate:	Uncoated
Protocol:	CDP-DNA standards or unknown DNA samples are first heat denatured before being adsorbed onto a 96-well DNA high-binding plate. The CPDs present in the sample or standard are probed with an anti- CPD antibody, followed by an HRP conjugated secondary antibody. The CPD content in an unknown sample is determined by comparing with a standard curve that is prepared from predetermined CPD- DNA standards.

Application Details

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: Immediately before use dilute the Anti-CPD Antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.
Assay Procedure:	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 CPD 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 CPD-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-CPD 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. 5
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.

Handling

Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, aliquot and store the Reduced DNA and CPD-DNA Standards at -20°C to avoid multiple freeze/thaw cycles. Store all other components at 4°C.

Publications

Product cited in:

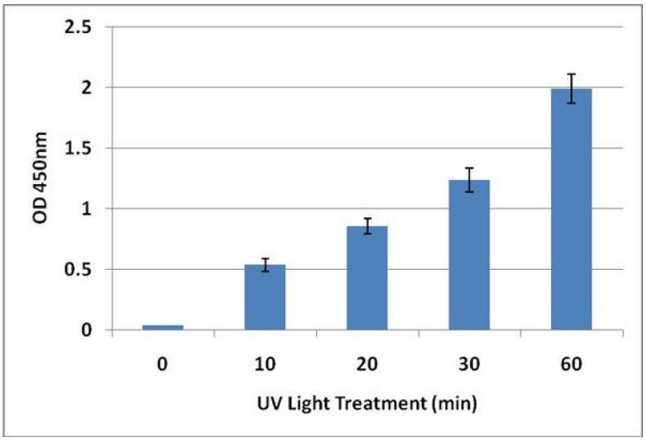
Cui, Li, Han, Wang, Wang, Ding, Zhang, Yan: "The Stress-responsive Gene ATF3 Mediates Dichotomous UV Responses by Regulating the Tip60 and p53 Proteins." in: **The Journal of biological chemistry**, Vol. 291, Issue 20, pp. 10847-57, (2016) (PubMed).

Rimann, Bono, Annaheim, Bleisch, Graf-Hausner: "Standardized 3D Bioprinting of Soft Tissue Models with Human Primary Cells." in: **Journal of laboratory automation**, (2015) (PubMed).

Donninger, Clark, Rinaldo, Nelson, Barnoud, Schmidt, Hobbing, Vos, Sils, Clark: "The RASSF1A tumor suppressor regulates XPA-mediated DNA repair." in: **Molecular and cellular biology**, Vol. 35, Issue 1, pp. 277-87, (2014) (PubMed).

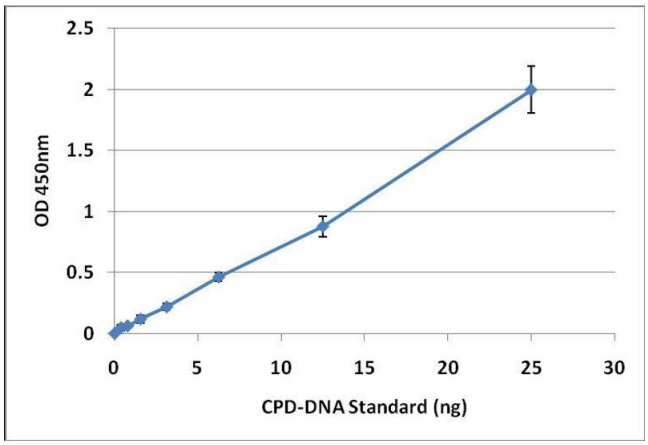
Zirkin, Davidovich, Don: "The PIM-2 kinase is an essential component of the ultraviolet damage response that acts upstream to E2F-1 and ATM." in: **The Journal of biological chemistry**, Vol. 288, Issue 30, pp. 21770-83, (2013) (PubMed).

Burgess, Richardson, Anderson, Salaun, Graham, Gray: "Nuclear relocalisation of cytoplasmic poly(A)-binding proteins PABP1 and PABP4 in response to UV irradiation reveals mRNA-dependent export of metazoan PABPs." in: **Journal of cell science**, Vol. 124, Issue Pt 19, pp. 3344-55, (2011) (PubMed).



Cellular Assay

Image 1. DNA Damage Induced by UV Light. Calf thymus DNA at 0.2 mg/mL was exposed to UV light inside a cell culture hood for the time indicated. The CPD levels in 40 ng denatured DNA samples were determined as described in the Assay Protocol for #STA-322.



ELISA

Image 2. CPD-DNA Standard Curve