COSMO BIO CO., LTD.
Inspiration for Life Science

Instruction manual

ELISA kit for measuring
UV-induced DNA damage

High Sensitivity 6-4PP/ (6-4)Photoproducts ELISA kit (TMB)

(with mAb clone 64M-2)

Catalog Number : NM-MA-K004(96 tests)

For research use only, Not for diagnostic use.

- Please read this manual thoroughly before use -

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INTRODUCTION

Prolonged exposure to solar UV radiation may result in harmful acute and chronic effects to the skin (including skin cancers), eye, and immune system. These harmful effects appear to be closely related to UV-induced DNA damage. The major types of DNA damage induced by solar UV radiation are cyclobutane pyrimidine dimers (CPDs), (6-4) photoproducts (6-4PPs), and Dewar photoproducts (DewarPPs), which are formed between adjacent pyrimidine nucleotides on the same DNA strand. These helix-distorting DNA lesions are repaired exclusively by a nucleotide excision repair system in humans. To better study molecular events surrounding UV-induced DNA damage and repair, Mori *et al.* previously developed and characterized monoclonal antibody (mAb) specific for CPDs and mAb specific for 6-4PPs (1) while Matsunaga *et al.* developed and characterized mAb specific for DewarPPs (2). Three of these antibodies (CPDs: clone TDM-2; 6-4PPs: clone 64M-2; DewarPPs: clone DEM-1) continue to be cited frequently in the literature, often for use in ELISA by a recommended procedure.

This High Sensitivity (6-4)photoproducts (6-4PPs) ELISA Kit is the only commercially available ELISA utilizing anti-6-4PPs clone 64M-2 and has been optimized for high sensitivity detection of 6-4PPs in DNA purified from cultured cells or from skin epidermis. This ELISA detects 6-4PPs from dipyrimidines in all DNA sequence contexts (i.e., TT, TC, CT and CC). Thus, the availability and convenience of this ELISA Kit will contribute to further understanding molecular mechanisms involved in cellular responses to UV radiation and DNA damage with applications across many research fields including cancer research, photobiology, dermatology, ophthalmology, immunology, and cosmetics science.

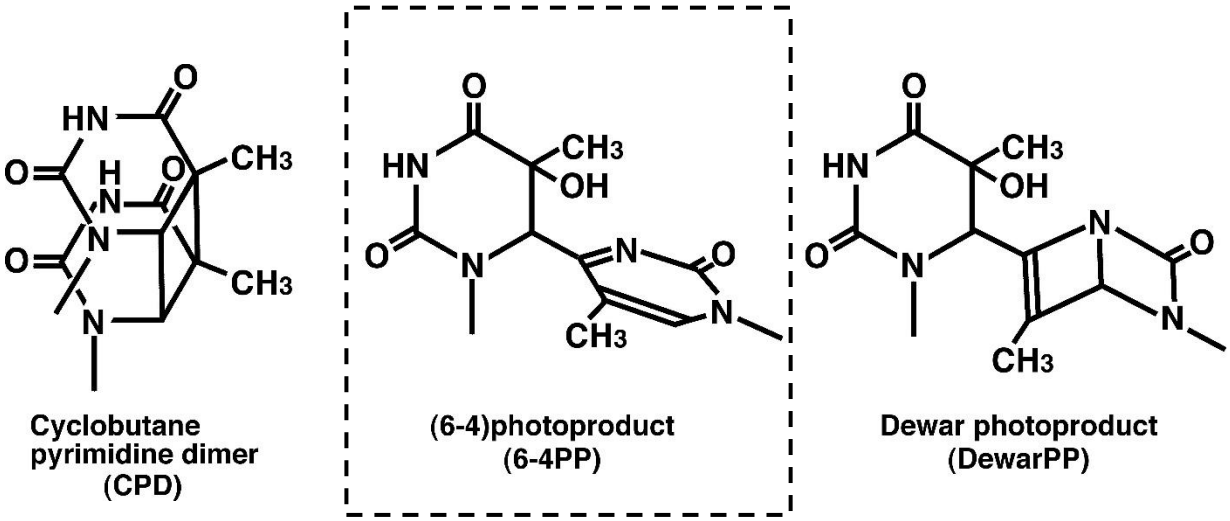

Figure 1: Structures of UV-induced DNA damage in thymine-thymine sequence

ASSAY PRINCIPLE

The format of this assay is ELISA with colorimetric detection. In brief, genomic DNA purified from UV-damaged cells is heat denatured and applied to microtiter wells pre-coated with protamine sulfate. 6-4PP specific monoclonal antibody clone 64M-2 (Cosmo Bio Cat. No. CAC-NM-DND-002) is then added to each well for thirty minutes and unbound antibody is removed by rinsing. The amount of 64M-2 antibody remaining in each well is then measured by sequential treatment of wells with biotinylated 2nd antibody, streptavidin-peroxidase, and *3,3',5,5'-tetramethylbenzidine* (TMB). The reaction between peroxidase, TMB produces a blue color, the strength of which is generally proportional to the amount of 64M-2 antibody remaining bound to the plate. The color development reaction is turned to yellow and stopped when sulfuric acid stop solution is added. The absorbance of each well at 450 nm is measured with a spectrophotometer.

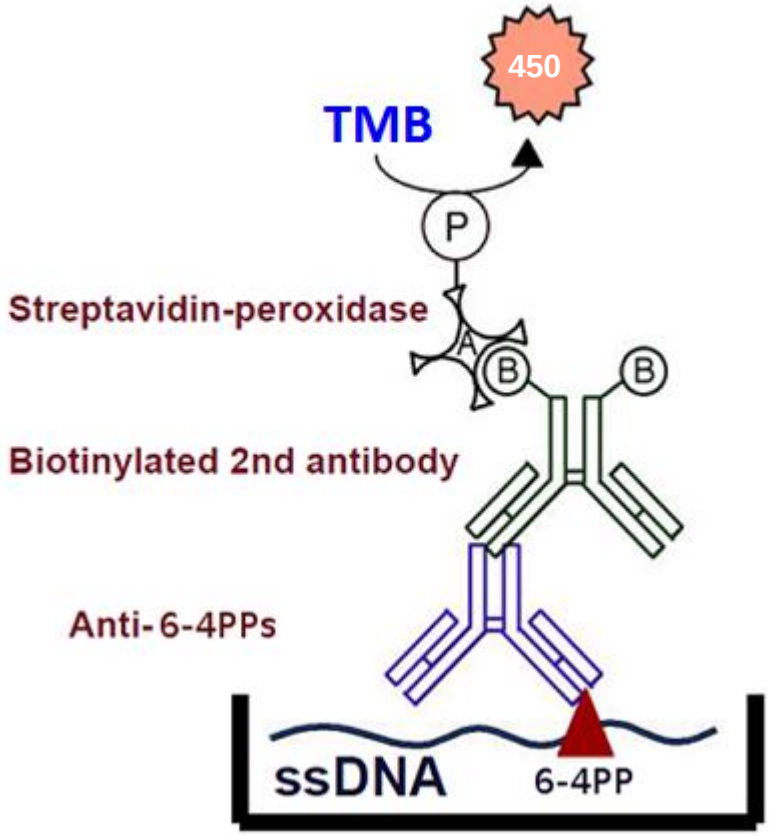

Figure 2: An ELISA for 6-4PPs

REACTIVITY

1) Anti-6-4PPs monoclonal antibody clone 64M-2 recognizes 6-4PPs on single-stranded DNA.
2) 64M-2 binds to 6-4PPs formed each dipyrimidine sequence context (TT, TC, CT and CC).
3) 64M-2 stably binds to 6-4PPs in DNA longer than eight bases.
4) 64M-2 binds to 6-4PPs in UV-irradiated DNA purified from a wide range of sources the prokaryote and eukaryote irradiated with UV.

KIT COMPONENTS

Item	Amount
ELISA plate precoated with protamine sulfate (12 x 8 well strips)	1 plate
Positive standard Calf thymus DNA, UVC irradiated (10 J/m^2)	1 vial (20 µg/mL, 500 µL)
Negative standard Calf thymus DNA, not irradiated	1 vial (20 µg/mL, 500 µL)
Assay Diluent Concentrate (10X)	1 vial (10 mL)
Wash Buffer Concentrate (20X)	2 x 15 mL vials
Blocking Reagent Concentrate (50X)	1 vial, lyophilized. Reconstitute with 400 µL purified water before use.
Anti-6-4PPs Monoclonal Antibody (clone 64M-2) (100X)	1 vial, lyophilized. Reconstitute with 150 µL purified water for a 100X working solution.
Biotinylated 2nd antibody (100X)	1 vial (150 µL)
Streptavidin-peroxidase (100X)	1 vial (150 µL)
TMB	1 vial (12 mL) Ready-to-use solution.
Stop Solution	1 vial (12 mL)
Plate Cover Film	3 covers
Instruction Manual	1 manual

MATERIALS TO BE SUPPLIED BY THE USERS

- DNA samples
- DNA Purification Kit (for sample preparation)
  Recommended: QIAamp Blood Kit (QIAGEN, Cat. No. 51104 or 51106)
- 100 °C Heating Block
- Ice bath (Crush ice)
- Purified water
- 10 µL - 1000 µL adjustable single channel micropipetters and disposable tips
- 50 µL - 150 µL adjustable multichannel micropipetters and disposable tips
- Reservoir for Wash Solution
- 1.5 mL tubes (for diluting samples)
- 15 mL or 50 mL tubes (for dilutions)
- 37°C Incubator (non-humidified)
- Absorbance microplate reader capable of reading 450 nm
- Vortex mixer
- Desktop centrifuge

STORAGE AND EXPIRATION

Unopened kit : 4 °C

Opened kit

Reconstituted solutions	: -20 °C
Reconstituted Antibody solution	: avoid thaw and freeze cycles
Positive and Negative Standards	: -20 °C
Other reagents	: 4 °C
ELISA plate precoated with protamine sulfate	: room temperature, protect from light

Expiration date

6 months from the shipping date.

PREPARATION OF REAGENTS

➢ ***Bring all reagents to room temperature (18-25 °C) before use.***

1. ELISA plate precoated with protamine sulfate

Bring to room temperature (18-25 °C) before use. Return unused wells to foil pouch.

2. Positive and Negative 6-4PP Standards

The concentration of the standard solutions is 20 µg/mL. Prepare *6-4PPs DNA Standard* solutions to a concentration of 4 µg/mL with 1X *Assay Diluent.*

3. Assay Diluent

Dilute 10 mL of *Assay Diluent* concentrate (10X) with 90 mL purified water to make 100 mL of *Assay Diluent* (1X).

4. Wash Buffer

Dilute 15 mL of *Wash Buffer* concentrate (20X) with 285 mL purified water to make 300 mL of *Wash Buffer* (1X).

5. Blocking Reagent

The *Blocking Reagent* is lyophilized. Reconstitute with 400 µL of purified water. Upon reconstitution, the solution is a 50X concentrate. Dilute 1:50 with *Assay Diluent* to prepare *Blocking Reagent* Working Solution.

6. Anti-6-4PPs

The *Anti-6-4PPs* antibody is lyophilized. Reconstitute with 150 µL of purified water. Upon reconstitution, the *Anti-6-4PPs* solution is a 100X concentrate. Dilute 1:100 with *Assay Diluent* to prepare *Anti-6-4PPs* Working Solution.

7. Biotinylated 2nd Antibody

The *Biotinylated 2nd Antibody* solution is a 100X concentrate. Dilute 1:100 with *Assay Diluent* to prepare *Biotinylated 2nd Antibody* Working Solution.

8. Streptavidin-peroxidase

The *Streptavidin-peroxidase* solution is a 100X concentrate. Dilute 1:100 with *Assay Diluent* to prepare *Streptavidin-Peroxidase* Working Solution.

9. TMB

The *TMB* is a ready-to-use substrate solution.

ASSAY PROTOCOLS

A. Cell culture and UV irradiation

1. Plate cells in 10 cm dishes and culture for one or two days.
2. Wash cells once with Dulbecco's PBS (DPBS) and irradiate with UV (e.g., 0, 2.5, 5, 7.5, 10 J/m^2 at 254 nm). To study DNA repair, irradiate cells with 10 J/m^2 and incubate for various amounts of time before harvesting (e.g., 3, 6, 12 hours) to allow repair.
3. Wash cells with 10 mL DPBS. Harvest by scraping cells from dish. Centrifuge at 10,000xg for 15 seconds at 4 °C.
4. Store cell pellets at –80 °C until ready for DNA isolation.

B. DNA isolation

5. Purify genomic DNA using a QIAamp Blood Kit (QIAGEN, Cat. No. 51104 or 51106) or similar. DNA concentrations are calculated by absorbance at 260 nm.

C. DNA sample coating to the ELISA plate precoated with protamine sulfate

6. Prepare sample DNA or 6-4PPs DNA Standard solutions to a concentration of 4 µg/mL with 1X *Assay Diluent.* Denature DNA solutions by heating to 100°C for 10 minutes, then chill rapidly in an ice bath for 15 minutes.
7. Apply 50 µL/well of denatured DNA solution to the ELISA plate wells precoated with protamine sulfate (duplicates recommended) and dry completely overnight by incubation at 37 °C.

D. DNA damage detection

8. Wash the DNA-coated plates 5 times with 150 µL/ well of *1X Wash Buffer*.
9. Add 150 µL/well *Blocking Reagent* Working Solution to each well to prevent non-specific binding of antibody.
10. Incubate 30 minutes at 37 °C.
11. Wash the plates 5 times with 150 µL/well of *1X Wash Buffer*.
12. Add 100 µL/well of *Anti-6-4PP* Working Solution and incubate 30 minutes at 37 °C.
13. Wash the plates 5 times with 150 µL/well of *1X Wash Buffer*.
14. Add 100 µL/well *Biotinylated 2nd Antibody* Working Solution and incubate 30 minutes at 37 °C.
15. Wash the plates 5 times with 150 µL/well of *1X Wash Buffer*.
16. Add 100 µL/well of *Streptavidin-Peroxidase* Working Solution and incubate 30 minutes at 37 °C.
17. Wash the plates 5 times with 150 µL/well of *1X Wash Buffer*.
18. Add 100 µL/well *TMB* to each well and incubate 30 minutes in dark place, at room temperature.
19. Add 100 µL/well *Stop Solution* to each well to stop enzyme reaction.
20. Mix gently and immediately determine the absorbance at 450 nm of each well using a spectrophotometer.

NOTES

- Do not mix or substitute reagents with those from other lots or sources.
- If a precipitate appear in *Assay Diluent* concentrate or *Wash Buffer* concentrate, warm it gently before use.
- TMB is highly reactive substrate solution; Avoid contacting the solution with any potential source of contamination.
- Prepare TMB solution just before use to prevent premature expiration by sunlight.
- Pour out needed amount of TMB solution into a plastic reservoir. Do not return the excess since it may cause degradation of the remaining content.

EXAMPLE OF RESULTS

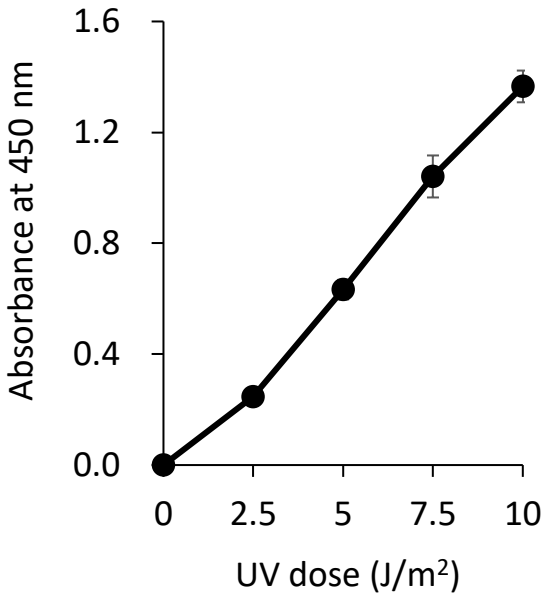

Figure 3: UV-induced 6-4PPs in DNA measured by ELISA

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More than 200 papers using 64M-2 antibodies have been published so far.

RELATED PRODUCTS

Product Name	Maker	Cat#
Anti cyclobutane pyrimidine dimers (CPDs) Monoclonal Antibody (Clone：TDM-2)	CAC	NM-DND-001
Anti (6-4) photoproducts (6-4PPs) Monoclonal Antibody (Clone：64M-2)	CAC	NM-DND-002
Anti Dewar photoproducts (DewarPPs) Monoclonal Antibody (Clone：DEM-1)	CAC	NM-DND-003
Anti Acetylaminofluorene(AAF)-DNA adducts Monoclonal Antibody (Clone：AAF-1)	CAC	NM-MA-001
High Sensitivity 6-4PP ((6-4)Photoproducts) ELISA kit ※with OPD	CSR	NM-MA-K002
High Sensitivity CPD (Cyclobutane Pyrimidine Dimer) ELISA kit Ver.2	CSR	NM-MA-K003
UVC irradiated DNA samples (0, 2.5, 5, 7.5, 10 J/m^2)	CSR	NM-MA-R010
PROTAMINE SULFATE COATED ELISA PLATE 96	CSR	NM-MA-P001
PROTAMINE SULFATE COATED ELISA PLATE 96 x 5	CSR	NM-MA-P002
PROTAMINE SULFATE COATED ELISA PLATE 96 x 10	CSR	NM-MA-P003
Anti XPA Monoclonal Antibody (Clone：A-2)	CAC	KUP-TM-M01
Anti XPA Monoclonal Antibody (Clone：5F12)	BAM	70-032
Anti XPF Monoclonal Antibody (Clone：19-16)	CAC	KUP-TM-M02
Anti XPG Monoclonal Antibody (Clone：G-26)	CAC	KUP-TM-M03
Anti ERCC1Monoclonal Antibody (Clone：E1-44)	CAC	KUP-TM-M04
Anti DDB1 Monoclonal Antibody (Clone：43233-3-1)	CAC	KUP-TM-M05

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ELISA kit for measuring
UV-induced DNA damage

High Sensitivity 6-4PP/ (6-4)Photoproducts ELISA kit (TMB)

(with mAb clone 64M-2)

Catalog Number : NM-MA-K004 (96 tests)

— FAQ & TROUBLESHOOTING —

FAQ

Q1. How to denature DNA.
A1. Heating for 10 min on Heating block (100 °C), then cooling for 15 min in ice bath (crush ice).

Q2. Can you measure the absolute amount of 6-4PP?
A2. No, but the relative amount of 6-4PP can be measured by this product.

Q3. How to reduce backgrounds.
A3. Try to dilute the concentration of Antibody.
Check the reasons in *TROUBLESHOOTING* No.3.

Q4. How to increase the signals.
A4. Try to increase the coating amount of DNA samples.
Check the reasons in *TROUBLESHOOTING* No.4.

TROUBLESHOOTING

1. Crystal substance was found in *Assay Diluent*.
➢ Warm and dissolve it in water bath.

2. Crystal substance was found in *Wash Buffer Diluent*.
➢ Warm and dissolve it in water bath.

3. High background absorbance.
➢ Possible reasons:

Purity of DNA sample is not enough.
The dilution of solutions is not correct.
The frequency of washing is not enough.
Plate was dried during the process.
Reaction time is too long.
The inside of plate is not clean.
Edge effect.

4. Low signal absorbance.
➢ Possible reasons:

Purity of DNA sample is not enough.
Sample DNA is not well denatured.
Sample DNA is not well immobilized on plate.
No 6-4PPs or very few 6-4PPs in samples.
The dilution of solutions is not correct.
Some reagents are missing.
Reaction time is too short.
The product has been expired.

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