



MONOCLONAL ANTIBODY

Anti-(6-4) photoproducts (6-4PPs)

Code No.	Clone	Subclass	Form	Quantity
NMDND002	64M-2	Mouse IgG2a κ	lyophilized	100 μ l

BACKGROUND:

DNA damage in cells exposed to ultraviolet (UV) radiation plays significant roles in cell-cycle arrest, activation of DNA repair, cell killing, mutation, and neoplastic transformation. The major types of DNA damage induced by UVB (280-315 nm, component of sunlight) and by UVC (200-280nm) are cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs), which are formed between adjacent pyrimidine nucleotides on the same strand of DNA. Approximately 70-80% of UV-induced DNA damage is CPDs and the remaining is 6-4PPs and Dewar isomer of 6-4PPs. These types of DNA lesions are repaired by nucleotide excision repair (NER) system in normal human cells. Mori *et al* (19) have established monoclonal antibodies specific for CPDs or 6-4PPs. These antibodies enable one to quantitate photoproducts in DNA purified from cultured cells or from the skin epidermis using an enzyme-linked immunosorbent assay (ELISA) and to visualize and measure photoproducts in DNA in cultured cells or the skin using indirect immunofluorescence (IIF). This technology would contribute to understanding of molecular mechanisms of cellular responses to UV and DNA damage in many research fields including cancer research, photobiology, dermatology, ophthalmology, immunology, and cosmetology.

SOURCE:

This hybridoma was established by fusion of mouse myeloma cells with Balb/c mouse splenocytes immunized with methylated BSA conjugated with calf thymus DNA which was irradiated with UVC. This hybridoma (clone 64M-2) culture supernatant was collected and precipitated with ice-cold ammonium sulfate. After centrifugation, the pellet dissolved in small volume of double-distilled water was dialysed against PBS. The dialysate was then lyophilized.

FORMULATION:

This antibody is lyophilized form.
Reconstitute with 100 μ l of distilled water. No preservative is contained.

STORAGE:

Lyophilized form (Before reconstitution) : store at -20°C.
Reconstituted form : store at -20°C.
After reconstitution, it is stable for at least 1 year when stored at -20°C
It should be divided into small quantity to avoid freezing and thawing.

REACTIVITY:

- 1) The antibodies bind to 6-4PPs in single-stranded DNA.
- 2) The antibodies bind to 6-4PPs formed in every dipyrimidine sequence (TT, TC, CT and CC).
- 3) The antibodies stably bind to 6-4PPs formed in oligonucleotides consisting of more than eight bases.

APPLICATIONS:

Immunocytochemistry; 1:300
ELISA; 1:1500
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.



SPECIES CROSS REACTIVITY:

The antibodies can bind to 6-4PPs in denatured DNA from all organisms from bacteria to human.

SELECTED REFERENCES:

- 1) Yamamoto, A., *et al.*, *DNA Repair*; **6**, 649-657 (2007).
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- 4) Sugawara, K., *et al.*, *Cell* **121**, 387-400 (2005).
- 5) Nishiwaki, Y., *et al.*, *J. Invest. Dermatol.* **122**, 526-532 (2004)
- 6) Imoto, K., *et al.*, *J. Invest. Dermatol.* **119**, 1177-7782 (2002)
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- 10) Otsoshi, E., *et al.*, *Cancer Res.* **60**, 1729-1735 (2000)
- 11) Nakagawa, A., *et al.*, *J. Invest. Dermatol.* **110**, 143-148 (1998)
- 12) Kobayashi, N., *et al.*, *J. Invest. Dermatol.* **110**, 806-810 (1998)
- 13) Komatsu, Y., *et al.*, *Nucleic Acids Res.* **25**, 3889-3894 (1997)
- 14) Nakane, H., *et al.*, *Nature* **377**, 165-168 (1995)
- 15) Todo, T., *et al.*, *Nature* **361**, 371-374 (1993)
- 16) Kobayashi, N., *et al.*, *J. Invest. Dermatol.* **101**, 685-689 (1993)
- 17) Potten, C.S., *et al.*, *Int. J. Radiat. Biol.* **63**, 313-324 (1993)
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- 19) Mori, T., *et al.*, *Photochem. Photobiol.* **54**, 225-232 (1991)

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

For research use only. Not for clinical diagnosis.

RELATED PRODUCTS:

NMDND001 Anti-cyclobutane pyrimidine dimers (TDM-2)
NMDND003 Anti-Dewar photoproducts (DEM-1)



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PROTOCOLS:

ELISA

A. The coating of microtiter plates by protamine sulfate

- 1) Prepare 0.003% protamine sulfate solution in distilled water and stir for 1 hour.
- 2) Distribute 50 μL / well of the solution to 96 well microtiter plates (Polyvinylchloride flat-bottom, Thermo, Cat. No. 2801, Milford, MA).
- 3) Incubate the plates at 37°C overnight and coat protamine sulfate on plates by drying completely.
- 4) Wash the plates three times with 100 μL / well of distilled water.
- 5) These plates can be stored for long times in dark.

B. Cell culture and UV irradiation

- 6) Plate cells in 10-cm dishes and culture one or two days.
- 7) Wash cells once by Dulbecco's PBS (DPBS) and irradiate cells with UV (for example ; 0, 5, 10, 15 J/m^2 of 254 nm UV). To study DNA repair, following UV irradiation with 15 J/m^2 , incubate cells for a variety of times (for example ; 1, 3, 8, 24 hours) to allow to repair.
- 8) Wash cells by 10 mL of DPBS and then cells were harvested by a cell scraper from the dishes and centrifuged at 10,000 x g for 15 seconds at 4 °C.
- 9) Cell pellets were stored at -80 °C until processing.

C. DNA isolation

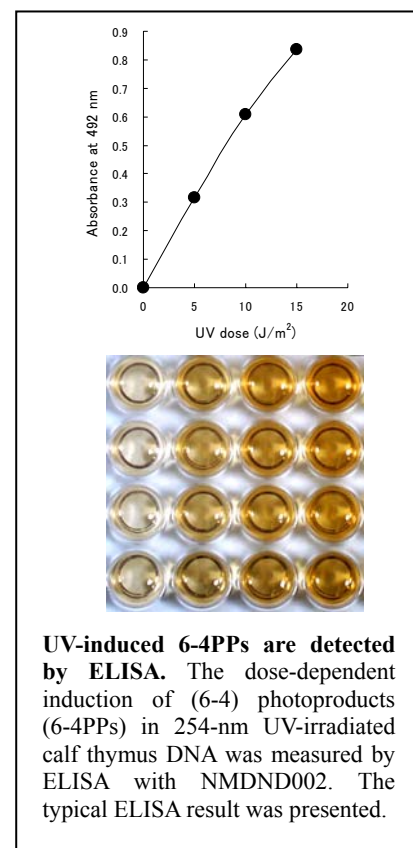
- 10) Genomic DNA was purified using a QIAamp Blood Kit (QIAGEN, Cat. No. 51104 or 51106). DNA concentrations were calculated from the absorbance at 260 nm.

D. DNA sample coating to the microtiter plates precoated with protamine sulfate

- 11) Prepare sample DNA solutions in PBS at the concentration of 4.0 μg / mL.
- 12) To denature DNA, heat DNA solutions in a hot plate at 100 °C for 10 minutes and chill rapidly in an ice bath for 15 minutes.
- 13) Distribute 50 μL / well of each denatured DNA solution to protamine sulfate precoated 96 well microtiter plates (use 4 wells for each sample) and dry completely overnight at 37 °C.

E. DNA Damage detection

- 14) Wash the DNA-coated plates 5 times with 150 μL / well PBS-T (0.05% Tween-20 in PBS).
- 15) Distribute 150 μL / well of 2% FBS in PBS to each well to prevent non-specific antibody binding.
- 16) Incubate 30 minutes at 37 °C.
- 17) Wash the plates 5 times with 150 μL / well of PBS-T.
- 18) Distribute 100 μL / well of 64M-2 antibodies diluted with PBS as suggested in the **APPLICATIONS** to each well and incubate 30 minutes at 37 °C.
- 19) Wash the plates 5 times with 150 μL / well of PBS-T.
- 20) Distribute 100 μL / well of 1:2000 Biotin-F(ab')₂ fragment of anti-mouse IgG (H+L) (Zymed, Cat. No. 62-6340) diluted with PBS to each well and incubate 30 minutes at 37 °C.
- 21) Wash the plates 5 times with 150 μL / well of PBS-T.
- 22) Distribute 100 μL / well of 1:10000 Peroxidase-Streptavidin (Zymed, Cat. No. 43-4323) diluted with PBS to each well and incubate 30 minutes at 37 °C.
- 23) Wash the plates 5 times with 150 μL / well of PBS-T.
- 24) Wash the plates once with 150 μL / well of Citrate-phosphate buffer (pH5.0) [Citric acid monohydrate 5.10 g, Na₂HPO₄ 7.30 g, Distilled water 1000 ml]. Keep the buffer solution in the plates until the next substrate solution is ready.
- 25) After throwing the buffer away, distribute 100 μL / well of the substrate solution [o-Phenylene diamine 8 mg, H₂O₂ (35%) 4 μL , Citrate-phosphate buffer (pH5.0) 20 ml] to each well and incubate 30 minutes at 37 °C.
- 26) Distribute 50 μL / well of 2M H₂SO₄ to each well and stop enzyme reaction.
- 27) After gentle mixing, determine the absorbance at 492 nm of each well by a spectrophotometer.





PROTOCOLS:

Immunofluorescence microscopy

A. Cell culture and UV irradiation

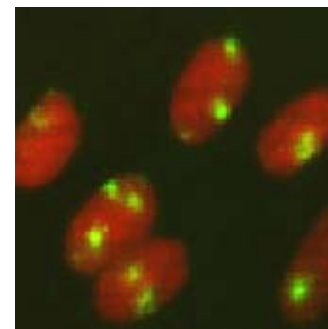
- 1) Culture the cells in the appropriate condition in 35-mm glass-bottom dishes (MatTek, Ashland, MA). (For example, inoculate 2×10^5 cells per dish, then incubate for one or two days in a CO₂ incubator.)
- 2) Wash cells once by DPBS and irradiate cells with UV [for example ; 10 J/m² of 254 nm UV for whole cell irradiation, or 100 J/m² of UV for local cell irradiation using a microfilter mask (1, 5, 6, 9)].

B. Cell fixation and permeabilization

- 3) Pour 1 mL of 4% formalin in PBS into each dish, and fix the cells for 10 minutes at room temperature.
- 4) Wash the cells 2 times with 2 mL of DPBS.
- 5) Pour 1 mL of 0.5% Triton X-100 in PBS, and permeabilize the cells for 5 minutes on ice.
- 6) Wash the cells 2 times with 2 mL of DPBS.
(When you want to stop the experiment at this stage, please do not freeze the samples. Instead, you should cover the samples with cold PBS overnight.)

C. Indirect Immunofluorescence

- 7) Pour 2 mL of 2M HCL and denature cellular DNA for 30 minutes at room temperature.
- 8) Wash the cells 5 times with 2 mL of PBS.
- 9) Pour 2mL of 20% FBS in PBS to prevent non-specific antibody binding.
- 10) Incubate 30 minutes at 37 °C with gentle shaking.
- 11) Wash the cells 5 times with 2 mL of PBS.
- 12) Add 70 µL of 64M-2 antibodies diluted with PBS containing 5% FBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at 37 °C with shaking (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 13) Wash the cells 5 times with 2 mL of PBS. (Subsequent steps must be done in the dark.)
- 14) Add 70 µL of 1:100 Alexa Fluor 594-F(ab')₂ fragment of anti-mouse IgG (H+L) (Molecular Probes, Cat. No. A-11020) diluted with PBS containing 5% FBS and incubate for 30 minutes at 37 °C with shaking.
- 15) Wash the cells 5 times with 2 mL of PBS.
- 16) Add 70 µL of 0.05 µg/ mL DAPI in PBS and incubate for 5 minutes at 37 °C with shaking.
- 17) Wash the cells 5 times with 2 mL of PBS.
- 18) Promptly add 20 µL of Vectashield mounting medium (Vector, Cat. No. H-1000) onto the cells, then put a cover slip on them.



Fluorescent image of localized 6-4PPs in normal human fibroblasts. Cells were cultured in a 35-mm glass-bottom dish for 24 hours. Immediately after micropore UV irradiation (100 J/m²), cells were fixed and permeabilized. After denaturation of DNA, 6-4PPs (yellow) were visualized using immunofluorescence with NMDND002. Nuclear DNA (red) was counterstained with propidium iodide. A filter with 3-µm pores was used.

041206-1



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