COSMO BIO CO., LTD.
Inspiration for Life Science

MONOCLONAL ANTIBODY

Anti-Dewar photoproducts (DewarPPs)

Code No.	Clone	Subclass	Form	Quantity
NMDND003	DEM-1	Mouse IgG1 λ	lyophilized	100 µl

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 solar UV radiation are cyclobutane pyrimidine dimers (CPDs), (6–4) photoproducts (6-4PPs), and Dewar valence isomers of 6-4PPs (Dewar photoproducts; DewarPPs), which are formed between adjacent pyrimidine nucleotides on the same strand of DNA. DewarPPs are produced by the photoisomerization of 6-4PPs by solar UV radiation with the highest efficiency around 325 nm. These helix-distorting DNA lesions are repaired by nucleotide excision repair (NER) system in normal human cells. Matsunaga *et al* (9) have established monoclonal antibodies specific for DewarPPs. The 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 using indirect immunofluorescence (IIF). This technology would contribute to understanding of molecular mechanisms of cellular responses to DewarPPs 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 and then with 313 nm UV. This hybridoma (clone DEM-1) culture supernatant was collected and precipitated with ice-cold ammonium sulfate. After centrifugation, the pellet dissolved in small volume of double-distilled water was dialyzed against PBS. The dialysate was then lyophilized.

FORMULATION:

This antibody is lyophilized form.
Reconstitute with 100 µl 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 DewarPPs in single-stranded DNA.
2) The antibodies bind to DewarPPs formed in TC, TT and CC dipyrimidine sequences.
3) The antibodies stably bind to DewarPPs formed in oligonucleotides consisting of more than eight bases.

APPLICATIONS:

Immunocytochemistry; 1:300
ELISA; 1:10000
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Flow cytometry; Not tested

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

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SPECIES CROSS REACTIVITY:

The antibodies can bind to DewarPPs in denatured DNA from all organisms from bacteria to human.

SELECTED REFERENCES:

1) Douki, T. and Cadet. J, *Biochemistry* **40**, 2495-2501 (2001).
2) Douki, T., *et al., J. Biol. Chem.*, **275**, 11678-11685 (2000).
3) Lee, J.H., *et al., Proc. Natl. Acad. Sci. U.S.A.*, **97**, 4591-4596 (2000).
4) Perdiz, D., *et al., J. Biol. Chem.* **275**, 26732-26742 (2000).
5) Kobayashi, N., *et al., J. Biochem.* **123**, 182-188 (1998)
6) Clingen, P.H., *et al., Photochem. Photobiol.* **61**, 163-170 (1995)
7) Clingen, P.H., *et al., Cancer Res.* **55**, 2245-2248 (1995)
8) Chadwick, C.A., *et al., J. Photochem. Photobiol. B.* **28**, 163-170 (1995)
9) Matsunaga, T., *et al., Photochem. Photobiol.* **57**, 934-940 (1993)
10) Matsunaga, T., *et al., Photochem. Photobiol.* **54**, 403-410 (1991)
11) Mitchell D.L. *Mutat. Res.*, **194**, 227-237 (1988).

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 Acetylaminofluorene(AAF)-DNA adducts Monoclonal Antibody (Clone : AAF-1)	CAC	NM-MA-001
High Sensitivity 6-4PP ELISA kit	CSR	NM-MA-K002
High Sensitivity CPD ELISA kit Ver.2	CSR	NM-MA-K003
High Sensitivity 6-4PP ELISA kit (TMB)	CSR	NM-MA-K004
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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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 Scientific, Cat. No. 2801, Milford, MA).
3) Incubate the plates at 37ºC 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. Solar UV-irradiation on DNA

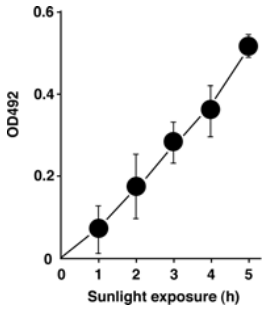
6) Prepare calf thymus DNA solution in H_2O at the concentration of 350 µg/mL.
7) Distribute 2 mL of DNA solution to each 35 mm dish on ice.
8) Expose ice-cold DNA solutions to sunlight for 0 - 5 hours on a fair day.
9) Store DNA solutions at -20 ºC in dark.

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

10) Calculate DNA concentrations from the absorbance at 260 nm.
11) Prepare sample DNA solutions in PBS at the concentration of 10 µg / mL.
12) Distribute 50 µL / well of each double-stranded DNA solution to protamine sulfate precoated 96 well microtiter plates (use 4 wells for each sample) and dry completely overnight at 37 ºC.

D. DNA Damage detection

13) Wash the DNA-coated plates 5 times with 150 µL/ well PBS-T (0.05% Tween-20 in PBS).
14) Distribute 150 µL / well of 2M HCL to each well to denature DNA (better than heat denature in this case).
15) Incubate 30 minutes at room temperature.
16) Wash the plates 5 times with 150 µL/ well of PBS-T.
17) Distribute 150 µL / well of 2% FBS in PBS to each well to prevent non-specific antibody binding and incubate 30 minutes at 37 ºC.
18) Wash the plates 5 times with 150 µL/ well of PBS-T.
19) Distribute 100 µL / well of DEM-1 antibodies diluted with PBS as suggested in the **APPLICATIONS** to each well and incubate 30 minutes at 37 ºC.
20) Wash the plates 5 times with 150 µL/ well of PBS-T.
21) Distribute 100 µL / well of 1:10000 Peroxidase-Streptavidin (Life Technologies, Cat. No. 62-6540) diluted with PBS to each well and incubate 30 minutes at 37 ºC.
22) Wash the plates 5 times with 150 µL/ well of PBS-T.
23) Distribute 100 µL / well of 1:10000 Peroxidase-Streptavidin (Life Technologies, Cat. No. 43-4323) diluted with PBS to each well and incubate 30 minutes at 37 ºC.
24) Wash the plates 5 times with 150 µL/ well of PBS-T.
25) Wash the plates once with 150 µL/ well of Citrate-phosphate buffer (pH5.0) [Citric acid monohydrate 5.10 g, Na_2HPO_4 7.30 g, Distilled water 1000 ml]. Keep the buffer solution in the plates until the next substrate solution is ready.
26) After throwing the buffer away, distribute 100 µL / well of the substrate solution [*o*-Phenylene diamine 8 mg, H_2O_2 (35%) 4 µl, Citrate-phosphate buffer (pH5.0) 20 ml] to each well and incubate 30 minutes at 37 ºC.
27) Distribute 50 µL / well of 2M H_2SO_4 to each well and stop enzyme reaction.
28) After gentle mixing, determine the absorbance at 492 nm of each well by a spectrophotometer.



Solar UV-induced DewarPPs are detected by ELISA. The exposure-dependent induction of Dewar photoproducts (DewarPPs) in solar UV-irradiated calf thymus DNA was measured by ELISA with NMDND003. The typical ELISA result was presented.

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PROTOCOLS:

Immunofluorescence microscopy

A. Cell culture and solar UV irradiation

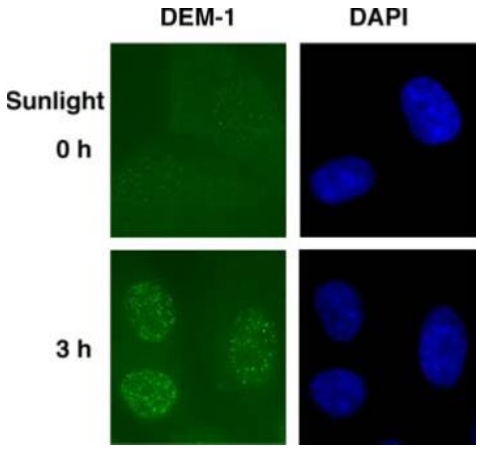
1) Culture the cells in DMEM (with HEPES buffer, no phenol red, #21063, Invitrogen, Carlsbad, CA) containing 10% FBS in 35-mm glass-bottom dishes (MatTek, Ashland, MA) in the appropriate condition. (For example, inoculate 2x105 cells per dish, then incubate one or two days in a CO2 incubator.)
2) Place the dishes on ice and expose cells to sunlight for 3 hours on a fair day.

B. Cell fixation and permeabilization

3) Wash the cells 2 times with 2 mL of Dulbecco's PBS (DPBS).
4) Pour 1 mL of 4% formalin in PBS [dilute 10% formalin solution, neutral buffered (e.g. Sigma-Aldrich, Cat. No. HT501128) with DPBS] into each dish, and fix the cells for 10 minutes at room temperature.
5) Wash the cells 2 times with 2 mL of DPBS.
6) Pour 1 mL of 0.5% Triton X-100 in PBS, and permeabilize the cells for 5 minutes on ice.
7) 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

8) Pour 2 mL of 2M HCL and denature cellular DNA for 30 minutes at room temperature.
9) Wash the cells 5 times with 2 mL of PBS.
10) Pour 2mL of 20% FBS in PBS to prevent non-specific antibody binding.
11) Incubate 30 minutes at 37 ºC with gentle shaking.
12) Wash the cells 5 times with 2 mL of PBS.
13) Add 70 µL of DEM-1 antibodies diluted with PBS containing 5% FBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at 37 ºC with shaking (Optimization of antibody concentration or incubation condition is recommended if necessary.)
14) Wash the cells 5 times with 2 mL of PBS. (Subsequent steps must be done in the dark.)
15) Add 70 µL of 1:200 Alexa Fluor 488-F(ab')2 fragment of anti-mouse IgG (H+L) (Life Technologies, Cat. No. A-11017) diluted with PBS containing 5% FBS and incubate for 30 minutes at 37 ºC with shaking.
16) Wash the cells 5 times with 2 mL of PBS.
17) Add 70 µL of 0.05 µg/ mL DAPI in PBS and incubate for 5 minutes at 37 ºC with shaking.
18) Wash the cells 5 times with 2 mL of PBS.
19) 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 DewarPPs in normal human fibroblasts. Cells were cultured in a 35-mm glass-bottom dish for 24 hours. Immediately after solar UV irradiation for 3 hours or mock irradiation, cells were fixed and permeabilized. After denaturation of DNA, DewarPPs (yellow) were visualized using immunofluorescence with NMDND003. Nuclear DNA (blue) was counterstained with DAPI.

CAUTION:

Please optimize the condition for your own experiment when you use the reagents not mentioned in the **PROTOCOLS** above.

Version#:5-170327

For research use only. Not for clinical diagnosis.

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