



Powerful tools in studying DNA damage and its biological effects

Antibodies against DNA Damage

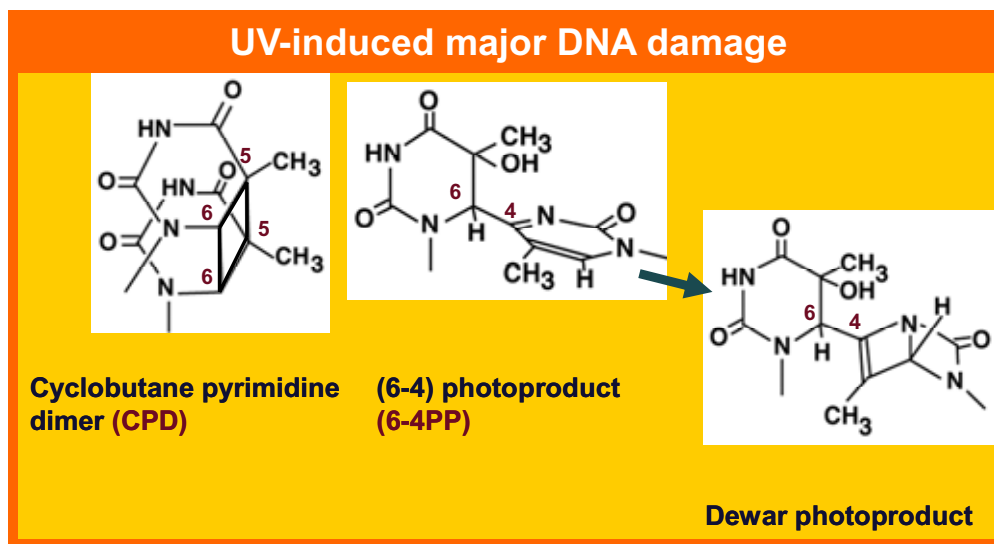
Anti Cyclobutane Pyrimidine Dimers (CPDs) [Clone : TDM-2]

Anti (6-4) photoproducts (6-4 PPs) [Clone : 64M-2]

Anti Dewar photoproducts (Dewar PPs) [Clone : DEM-1]

Prolonged exposure to solar UV radiation may result in acute and chronic health effects on the skin, eye, and immune system, including skin cancers. These harmful effects are suggested to deeply relate to 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 strand of DNA. These helix-distorting DNA lesions are repaired exclusively by nucleotide excision repair system in humans. Mori *et al.* have developed and characterized monoclonal antibodies specific for CPDs or 6-4PPs (1). Matsunaga *et al.* have established and characterized monoclonal antibodies against DewarPPs (2). 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. Thus, 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.

- (1) Toshio Mori, Misa Nakane, Tsuyoshi Hattori, Tsukasa Matsunaga, Makoto Ihara, Osamu Nikaido, Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or (6-4) photoproduct from the same mouse immunized with ultraviolet-irradiated DNA. *Photochem. Photobiol.*, 54: 225-232 (1991).
- (2) Tsukasa Matsunaga, Yuri Hatakeyama, Michi Ohta, Toshio Mori and Osamu Nikaido, Establishment and characterization of a monoclonal antibody recognizing the Dewar isomers of (6-4) photoproducts. *Photochem. Photobiol.*, 57: 934-940 (1993).



Features

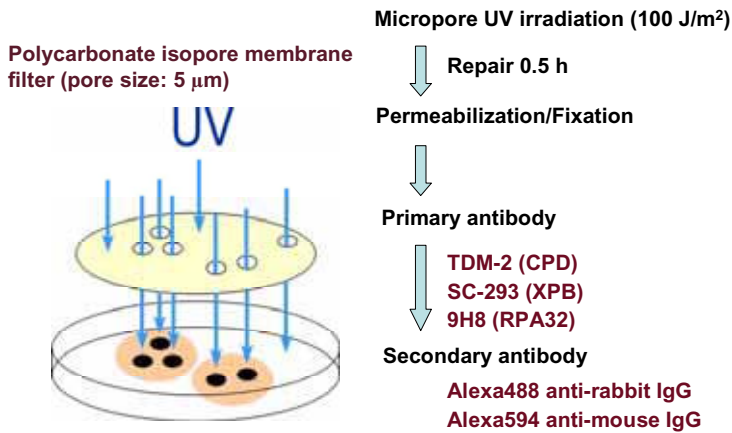
- Very specific for the particular UV-induced DNA lesion
- Research applications include ELISA, IF, and IHC
- Useful for research in DNA damage and repair
- Visualizes the DNA damage repair process
- Applicable to a broad range of research fields including cancer research, photobiology, dermatology, ophthalmology, immunology, and cosmetology

Description	Cat#	Host	Clone	Application	Size	Price
Anti CPDs	CAC-NMDND001	Mouse	TDM-2	ELISA/IC	1vial	\$420
Anti 6-4 PPs	CAC-NMDND002	Mouse	64M-2	ELISA/IC	1vial	\$420
Anti Dewar PPs	CAC-NMDND003	Mouse	DEM-1	ELISA/IC	1vial	\$420

Application

Immunocytochemistry

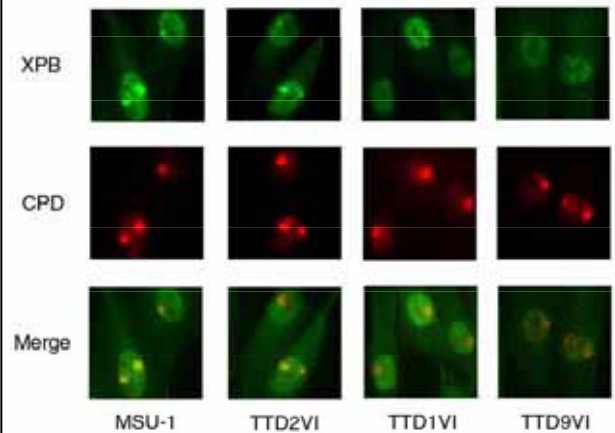
In situ visualization of XPB (TFIIH) and RPA at CPD sites after micropore UV irradiation



Katsumi et al., *J. Invest. Dermatol.* 117: 1156-1161, 2001

The technique of micropore UV irradiation combined with fluorescent antibody labeling is very powerful for examining whether a protein of interest is recruited to the sites of UV-induced DNA damage. Micropore UV irradiation induces UV-damage at localized areas of nuclei using a polycarbonate isopore membrane filter. The polycarbonate blocks UV radiation, and cells are exposed only through the 5 μm pores of the filter. 0.5 h after micropore UV irradiation, cells were fixed and Immunofluorescent double staining for DNA damage and repair protein were performed.

In Situ Visualization of XPB and CPD 30 min after micropore UV irradiation

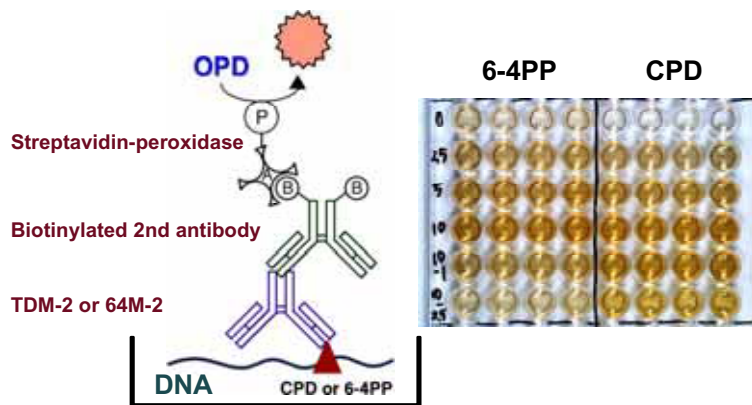


Nishiwaki et al., *J. Invest. Dermatol.* 122: 526-532, 2004

Cells were doubly stained for XPB and for CPD 0.5 h after local UV irradiation. In normal MSU-1 cells, XPB foci overlapped with the corresponding CPD foci, indicating that XPB is quickly recruited to the sites of DNA damage for repair. In contrast, no or less bright XPB foci at the DNA damage sites were observed in repair deficient TTD cell lines.

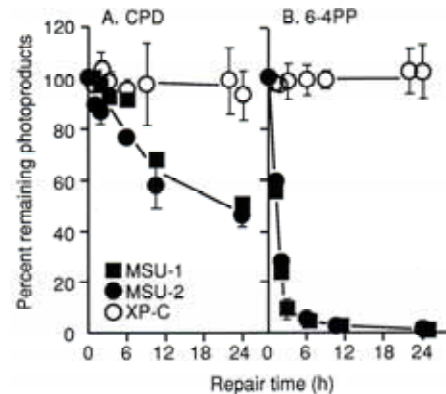
ELISA

The sensitive ELISA for measuring UV-induced DNA damage



Genomic DNA is purified from UV-damaged cells and denatured DNA is coated on wells of 96-well plate. The binding of TDM-2 or 64M-2 to DNA damage is detected by sequential treatment with biotinylated 2nd antibody and streptavidin-peroxidase. Then, the absorbance of colored products derived from OPD is measured at 492 nm.

Quantification of DNA damage repair by ELISA



Nakagawa et al., *J. Invest. Dermatol.* 110: 143-148, 1998

Normal human cells repair 90% of the initial 6-4PP within 3 h after UV irradiation, while they remove 50% of the initial CPD within 24 h. Both damage are repaired by the same nucleotide excision repair (NER) pathway, but 6-4PP forms bigger distortion in DNA than CPD does, resulting in much more efficient repair. In contrast, repair deficient XP-C cells can not repair both damage at all.



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