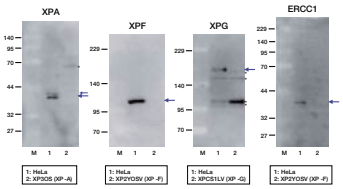
Antibodies against Nucleotide excision repair (NER) factors

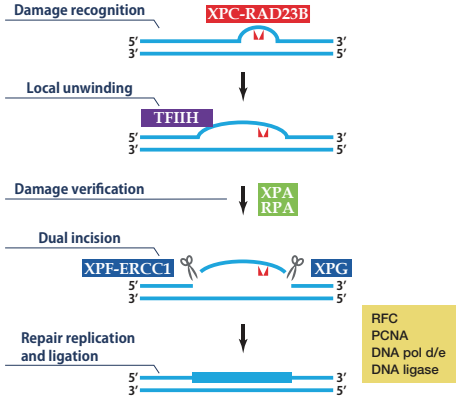
Anti XPA [Clone : A-2]
Anti XPF [Clone : 19-16]
Anti XPG [Clone : G-26]
Anti ERCC1 [Clone : E1-44]

Nucleotide excision repair (NER) is a major repair system for removing a variety of DNA lesions including UV-induced cyclobutane pyrimidine dimer and (6-4) photoproduct as well as chemical-induced bulky base adducts. Defects in the NER system give rise to xeroderma pigmentosum (XP), an autosomal recessive disease characterized by a predisposition to skin cancer and in some cases neurological abnormalities. The early process of human NER, from damage recognition to dual incision (removal of damage-containing oligonucleotides), is accomplished by six core NER factors, XPC-RAD23B, TFIIH, XPA, RPA, XPF-ERCC1 and XPG *in vitro*.

Western blot



Current Model for the Dual Incision Process of NER



Description	Host	Clone	Application	Cat. No.	Quantity
Anti XPA	XPA has an ability to bind to DNA with some preference to damaged DNA and interacts with most of other NER factors. XPA appears to be involved in a proper assembly of preincision complex and verification of damaged DNA strand.				
	Mouse	A-2	WB	CAC-KUP-TM-M01	100 µl
	Mouse	5F12	WB / ELISA	BAM-70-031	50 µg
	Mouse	5F12	WB / ELISA	BAM-70-032	250 µg
Anti XPF	XPF harbors a nuclease domain and forms a stable complex with ERCC1. The ERCC1-XPF complex has a unique ability to make a nick on the DNA strand which makes the transition from duplex to single-stranded DNA in the 5' to 3' direction. In the NER process, ERCC1-XPF is responsible for 5'-incision at a dual incision step.				
	Mouse	19-16	WB / IF	CAC-KUP-TM-M02	100 µl
Anti XPG	XPG is a structure-specific endonuclease with an opposite polarity to ERCC1-XPF and makes a nick on the DNA strand which makes the transition from single-stranded to duplex DNA in the 5' to 3' direction. In the NER process, XPG is responsible for 3'-incision at a dual incision step.				
	Mouse	G-26	WB	CAC-KUP-TM-M03	100 µl
Anti ERCC1	ERCC1 forms a stable complex with XPF and the heterodimer has an ability to make a nick on the DNA strand which makes the transition from duplex to single-stranded DNA in the 5' to 3' direction. In the NER process, ERCC1-XPF complex is responsible for 5'-incision at a dual incision step.				
	Mouse	E1-44	WB	CAC-KUP-TM-M04	100 µl

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Monoclonal Antibodies against DNA Damage

Powerful tools for studying DNA damage and its biological effects

Monoclonal antibodies against UV-induced DNA Damage

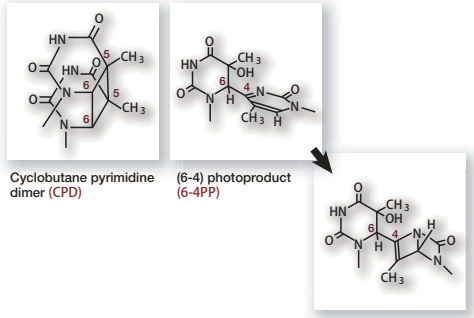
Anti Cyclobutane Pyrimidine Dimers (CPDs) [Clone : TDM-2]
Anti (6-4) photoproducts (6-4PPs) [Clone : 64M-2]
Anti Dewar photoproducts (DewarPPs) [Clone : DEM-1]

Prolonged exposure to solar UV radiation may result in acute and chronic health effects to the skin, eye, and immune system, including skin cancers. These harmful effects are suggested to be closely related 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 a nucleotide excision repair system in humans. Mori *et al.* have developed and characterized monoclonal antibodies specific for CPDs and for 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 will contribute to understanding the molecular mechanisms of cellular responses to UV light and DNA damage in many research fields including cancer research, photobiology, dermatology, ophthalmology, immunology, and cosmetology.

Features

- Highly specific for the target lesion
- Research applications include ELISA, IF and IHC
- Useful for research in DNA damage and repair
- Allows visualization of the DNA repair process
- Applicable to a broad range of research fields including cancer research, photobiology, dermatology, ophthalmology, immunology, and cosmetology

UV-induced major DNA damage



(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).

Description	Host	Clone	Application	Cat. No.	Quantity
Anti CPDs	Mouse	TDM-2	ELISA / IC	CAC-NM-DND-001	1 vial
Anti 6-4PPs	Mouse	64M-2	ELISA / IC	CAC-NM-DND-002	1 vial
Anti DewarPPs	Mouse	DEM-1	ELISA / IC	CAC-NM-DND-003	1 vial

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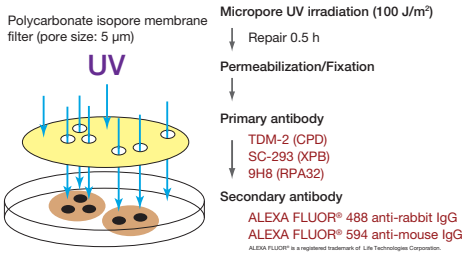
Monoclonal antibodies against UV-induced DNA Damage

Anti CPDs [Clone : TDM-2] Anti 6-4PPs [Clone : 64M-2] Anti DewarPPs [Clone : DEM-1]

Immunocytochemistry

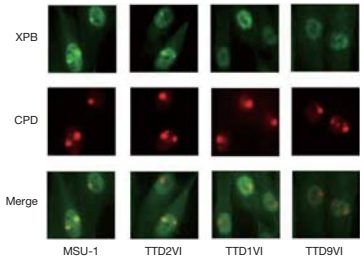
In situ visualization of XPB (TFIIH) and RPA at CPD sites after micropore UV irradiation



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

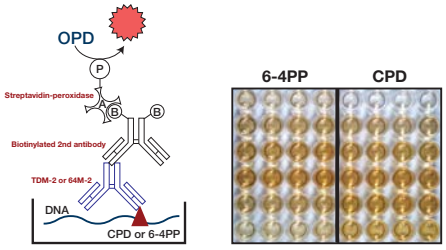


Nishiwaki *et. al.*, *J. Invest. Dermatol.* **122**: 526-532, 2004.

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.

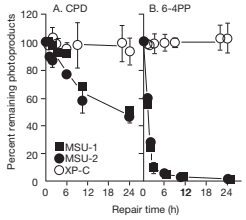
ELISA

A sensitive ELISA for measuring UV-induced DNA damage



Genomic DNA is purified from UV-damaged cells and denatured DNA is used to coat wells of a 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.

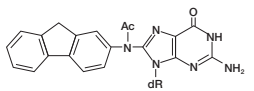
Normal human cells repair 90% of the initial 6-4PP within 3 h after UV irradiation, while they remove 50% of the initial CPD within 24 h. Both damage are repaired by the same nucleotide excision repair (NER) pathway, but 6-4PP forms bigger distortion in DNA than CPD does, resulting in much more efficient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

Anti Acetylaminofluorene-DNA Adducts Monoclonal Antibody

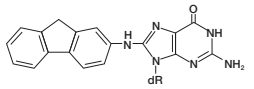
Anti AAF-DNA adducts [Clone : AAF-1]

DNA adducts in mammalian cells exposed to N-acetoxy-2-acetylaminofluorene (NA-AAF), an activated derivative of the potent carcinogen 2-AAF, play significant roles in cell killing, chromosome aberration, gene mutation and neoplastic transformation. NA-AAF binds covalently to guanine in the DNA of mammalian cells and produces three different DNA adducts. The C-8 adducts dG-C8-AAF and deacetylated dG-C8-AF account for the major portion of the DNA-bound products, while the minor N2 adduct dG-N2-AAF accounts for the remainder. The relative induction levels of the two major C-8 adducts vary among cell types. These adducts distort the DNA helix and therefore are repaired by nucleotide excision repair in human cells. Our AAF-1 antibodies bind most efficiently to dG-C8-AAF and less efficiently to dG-C8-AF in denatured DNA. The antibodies enable one to detect AAF-DNA adducts in DNA from cultured cells using an enzyme-linked immunosorbent assay (ELISA) and to visualize them in cultured cells or rodent tissues by immunofluorescence (IF). This technology will contribute to understanding of molecular mechanisms in AAF-related research fields including cancer research, anticancer research and toxicology.

AAF-DNA adducts recognized by AAF-1

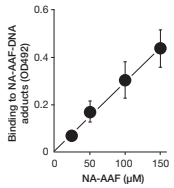


dG-C8-AAF



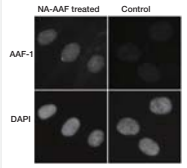
dG-C8-AF

The dose-dependent formation of NA-AAF-induced DNA adducts in human cells.



Cells were exposed to NA-AAF for 0.5 h and the formation of DNA adducts in denatured DNA (500 ng/well) was determined using a sensitive-direct-binding ELISA with AAF-1 (1/100).

Visualization of NA-AAF-induced DNA adducts in human cells.



Cells were exposed to 200 μM NA-AAF or solvent for 0.5 h. After permeabilization and fixation, DNA adducts were visualized by sequential treatment of AAF-1 (1/25) and ALEXA FLUOR® 488 goat anti-mouse IgG conjugate. Nuclear DNA was counterstained with DAPI.

Description	Host	Clone	Application	Cat. No.	Quantity
Anti AAF-DNA adducts	Mouse	AAF-1	ELISA / IC	CAC-NM-MA-001	1 vial

Useful for ELISA assays with DNA damage antibodies

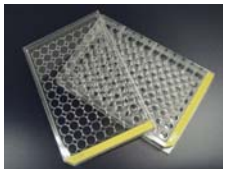
PROTAMINE SULFATE COATED ELISA PLATE

New

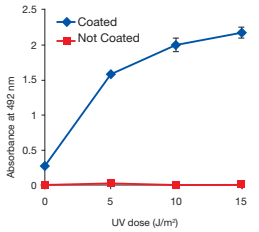
Protamine sulfate is a small cationic protein that binds to negatively charged DNA. Protamine sulfate coated wells capture sample DNA more efficiently; a critical step in the accurate and reproducible determination of DNA damage detection by ELISA.

- Steady DNA binding
- High signal detection of a small amount (low concentration) sample
- Room temperature preservation

* Plate seal 1 sheet



Protamine coating increases DNA-binding



UV- or mock-irradiated DNA (20 ng) was added to plates either coated, or not coated, with protamine sulfate. CPDs were then detected by ELISA using TDM-2 antibody. Protamine sulfate coated wells produced strong dose-dependent CPD signals whereas non-coated wells produced very poor signals.

Description	Cat. No.	Quantity
PROTAMINE SULFATE COATED ELISA PLATE 96	CSR-NM-MA-P001	1 plate
PROTAMINE SULFATE COATED ELISA PLATE 96×5	CSR-NM-MA-P002	5x1 plate
PROTAMINE SULFATE COATED ELISA PLATE 96×10	CSR-NM-MA-P003	10x1 plate