

Anti-Acetylaminofluorene-DNA adducts (Anti-AAF-DNA adducts)

BACKGROUND

DNA adducts in mammalian cells exposed to *N*-acetoxy-2-acetylaminofluorene* (NA-AAF), an activated derivative of the potent carcinogen 2-acetylaminofluorene (2-AAF), play significant roles in cell killing, chromosome aberration, sister-chromatid exchange, gene mutation and neoplastic transformation (1,2). NA-AAF binds covalently to guanine in the DNA of mammalian cells and produces three different DNA adducts. The C-8 adducts, *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-C8-AAF) and deacetylated *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF), account for the major portion of the DNA-bound products, while the minor N2 adduct, 3-(deoxyguanosin-N2-yl)-2-acetylaminofluorene (dG-N2-AAF), accounts for the rest of them. The relative induction levels of the two major C-8 adducts vary among cell types. These adducts distort the DNA helix as do UV-induced cyclobutane pyrimidine dimers (CPD), and therefore they are repaired by nucleotide excision repair in human cells. Iwamoto *et al* (3) have established monoclonal antibodies against dG-C8-AAF in denatured DNA. These antibodies enable one to detect dG-C8-AAFs in DNA from cultured cells using an enzyme-linked immunosorbent assay (ELISA) and to visualize them in cultured cells or rodent tissues using an immunofluorescence (IF). This technology would contribute to understanding of molecular mechanisms in AAF-related research fields including cancer research, anticancer research or toxicology.

*NA-AAF is available from NCI Chemical Carcinogen Reference Standards Repository (Midwest Research Institute, Kansas City, MO)

Product type	Primary antibody
Clone number	AAF-1
Isotype	IgG1 (kappa)
Host	Mouse
Source	The hybridoma was established by fusion of mouse myeloma cells with Balb/c mouse splenocytes immunized with NA-AAF-modified single-stranded DNA conjugated with methylated BSA. This hybridoma (clone AAF-1) culture supernatant was collected and precipitated with ammonium sulfate. After centrifugation, the pellet dissolved in small volume of double-distilled water was dialysed against PBS. The dialysate was then lyophilized.
Form	This antibody is lyophilized form. Reconstitute with 100 µl of distilled water. No preservative is contained.
Volume	100 uL(Lyophilized Powder)
Specificity	AAF-1 primarily binds to dG-C8-AAF in denatured DNA, although dG-C8-AF in DNA is also recognized with slightly less efficiency.
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 many times.

Application notes Recommended dilutions

- **Immunohistochemistry:** 1/25
- **ELISA:** 1/100

Other applications have not been tested.

Optimal dilutions/concentrations should be determined by the end user.

References

- 1) R.H. Heflich and R.E. Neft, Genetic toxicity of 2-acetylaminofluorene, 2-aminofluorene and some of their metabolites and model metabolites. *Mutation Res.* 318 (1994) 73-174.
- 2) E.Kriek, Fifty years of research on N-acetyl-2-aminofluorene, one of the most versatile compounds in experimental cancer research. *J. Cancer Res. Clin. Oncol.* 118 (1992) 481-489.
- 3) T. Iwamoto *et al.*, In situ detection of acetylaminofluorene-DNA adducts in human cells using monoclonal antibodies. *DNA Repair* 3 (2004) 1475-1482.

ANTIBODY CHARACTERIZATION

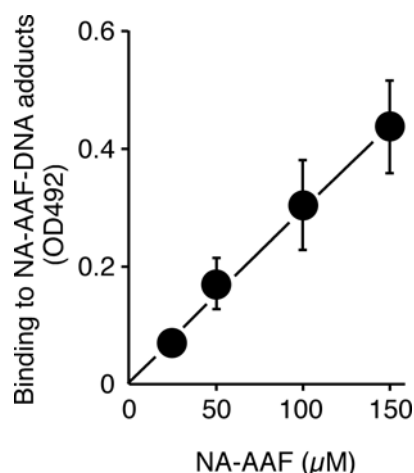


Fig.1 The dose-dependent formation of NA-AAF-induced DNA adducts in human cells.
Cells were exposed to NA-AAF for 0.5 h and the formation of DNA adducts in denatured DNA (500 ng/well) was determined using a sensitive-direct-binding ELISA with AAF-1 (1/100). (Details are shown in Ref. 3.)

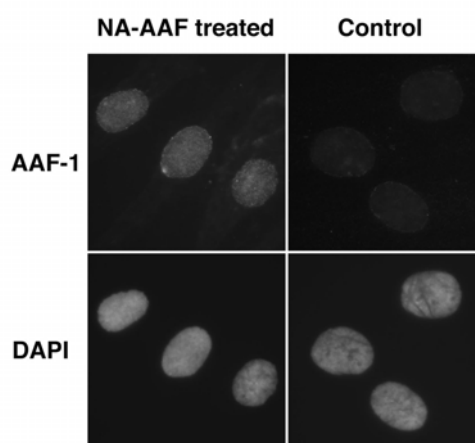


Fig.2 The formation of NA-AAF-induced DNA adducts in human cells.
Cells were exposed to 200 μM NA-AAF or solvent for 0.5 h. After permeabilization and fixation, DNA adducts were visualized by sequential treatment of AAF-1 (1/25) and Alexa Fluor 488 goat anti-mouse IgG conjugate. Nuclear DNA was counterstained with DAPI. (Details are shown in Ref. 3.)

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
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:A-2)	CAC	KUP-TM-M02
Anti XPG Monoclonal Antibody (Clone:A-2)	CAC	KUP-TM-M03
Anti ERCC1 Monoclonal Antibody (Clone:A-2)	CAC	KUP-TM-M04
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

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 μ L / 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 μ L / well of distilled water.
- 5) These plates can be stored for long times in dark.

B. Cell culture and NA-AAF exposure

- 6) Plate cells in 10-cm dishes and culture one or two days.
- 7) Prepare 100 mM NA-AAF in DMSO and expose cells to NA-AAF (for example ; 0, 25, 50, 100, 150 μ M) for 30 minutes at 37°C.
- 8) Wash cells twice by 10 mL of Dulbecco's PBS (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 10 μ g / 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 μ L / 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 adduct detection

- 14) Wash the DNA-coated plates 5 times with 150 μ L / well of PBS-T (0.05% Tween-20 in PBS).
- 15) Distribute 150 μ L / 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 μ L / well of PBS-T.
- 18) Distribute 100 μ L / well of AAF-1 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 μ L / well of PBS-T
- 20) Distribute 100 μ L / well of 1:2000 Biotin-F(ab')₂ fragment of anti-mouse IgG (H+L) (Rockland, Code 710-106-019) diluted with PBS to each well and incubate 30 minutes at 37°C.
- 21) Wash the plates 5 times with 150 μ L / well of PBS-T.
- 22) Distribute 100 μ L / 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 μ L / well of PBS-T.
- 24) Wash the plates once with 150 μ L / 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 μ L / well of the substrate solution [o-Phenylene diamine 8 mg, H₂O₂ (35%) 4 μ l, Citrate-phosphate buffer (pH 5.0) 20 ml] to each well and incubate 30 minutes at 37°C]
- 26) Distribute 50 μ L / 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 NA-AAF exposure

- 1) Culture the cells in the appropriate condition in 35-mm glass-bottom dishes (MatTek, Ashland, MA). (For example, inoculate 4×10^5 cells per dish, then incubate for one or two days in a CO₂ incubator.)
- 2) Prepare 100 mM NA-AAF in DMSO and expose cells to NA-AAF (for example; 0, 200 μ M) for 30 minutes at 37 °C. Wash cells 2 times with 2 mL of DPBS.

B. Cell fixation and permeabilization

- 3) Pour 1 mL of 0.5% Triton X-100 in PBS, and permeabilize the cells for 15 minutes on ice.
- 4) Wash the cells 2 times with 2 mL of DPBS.
- 5) Pour 2 mL of methanol-aceton (1 : 1) into each dish, and fix the cells for 10 minutes at -20 °C.
- 6) Dry the dishes at room temperature for 5-10 minutes.

C. Indirect Immunofluorescence

- 7) Add 70 μ L 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) Add 70 μ L 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 μ L of AAF-1 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 μ L of 1:100 Alexa Fluor 488-F(ab')₂ fragment of anti-mouse IgG (H+L) (Molecular Probes, Cat. No. A-11017) diluted with PBS containing 5% FBS and incubate for 30 minutes at 37 °C with shaking. Distribute 100 μ L / well of 1:10000 Peroxidase-Streptavidin (Zymed, Cat. No. 43-4323) diluted with PBS to each well and incubate 30 minutes at 37 °C.
- 15) Wash the cells 5 times with 2 mL of PBS.
- 16) Add 70 μ L of 0.05 μ g/ 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 μ L of Vectashield mounting medium (Vector, Cat. No. H-1000) onto the cells, then put a cover slip on them.
- 19) Observe fluorescence images by a fluorescence microscope.

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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.

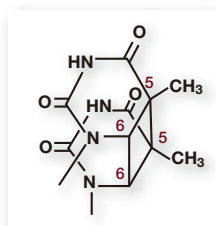
Features

- 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

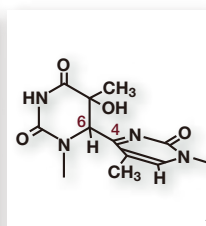
(1) Toshio Mori, Misa Nakane, Tsuyoshi Hattori, Tsukasa Matsunaga, Makoto Ihara, Osamu Nikaïdo, Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or (6-4) photoproduct from the same mouse immunized with ultraviolet-irradiated DNA. *Photochem. Photobiol.*, 54: 225-232 (1991).

(2) Tsukasa Matsunaga, Yuri Hatakeyama, Michi Ohta, Toshio Mori and Osamu Nikaïdo, Establishment and characterization of a monoclonal antibody recognizing the Dewar isomers of (6-4) photoproducts. *Photochem. Photobiol.*, 57: 934-940 (1993).

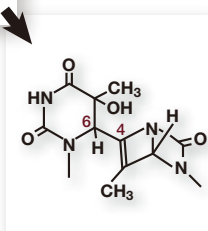
UV-induced major DNA damage



Cyclobutane pyrimidine dimer (CPD)



(6-4) photoproduct (6-4PP)



Dewar photoproduct (DewarPP)

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 DNA Damage

Monoclonal antibodies against UV-induced DNA Damage

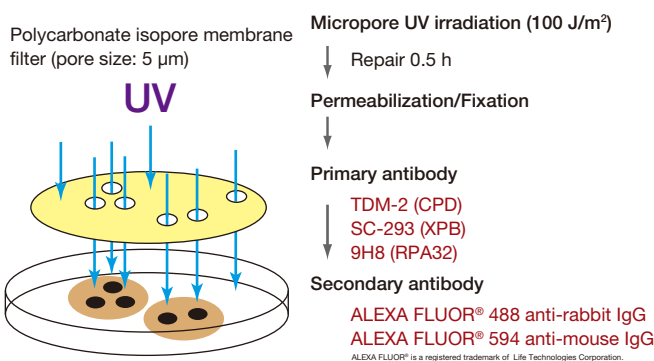
Anti CPDs [Clone : TDM-2]

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

Anti DewarPPs [Clone : DEM-1]

Immunocytochemistry

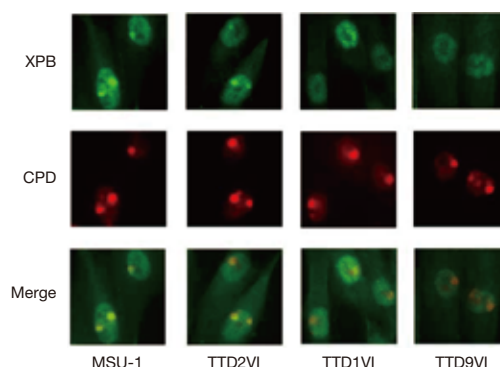
In situ visualization of XPB (TFIIH) and RPA at CPD sites after micropore UV irradiation



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 μ m 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

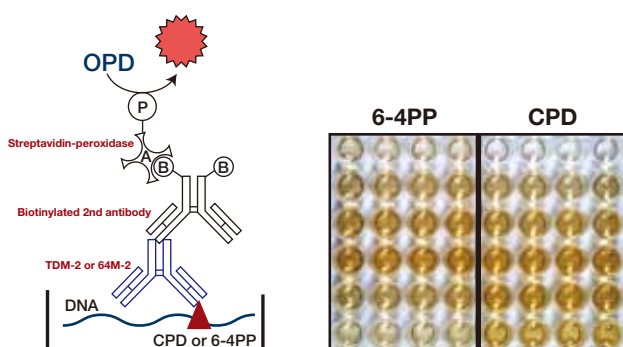


Nishiwaki et. al., *J. Invest. Dermatol.* 122: 526-532, 2004.

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.

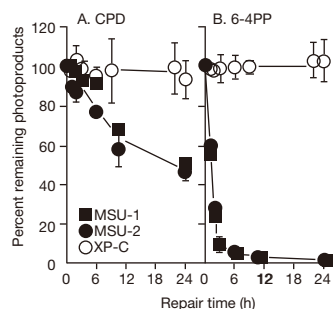
ELISA

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.

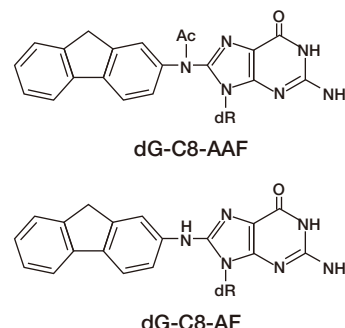
Normal human cells repair 90% of the initial 6-4PP within 3 h after UV irradiation, while they remove 50% of the initial CPD within 24 h. Both damage are repaired by the same nucleotide excision repair (NER) pathway, but 6-4PP forms bigger distortion in DNA than CPD does, resulting in much more efficient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

Anti Acetylaminofluorene-DNA Adducts Monoclonal Antibody

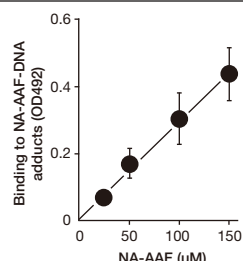
Anti AAF-DNA adducts [Clone : AAF-1]

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

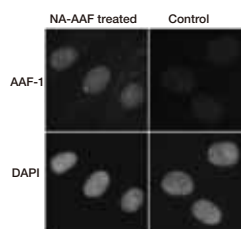


The dose-dependent formation of NA-AAF-induced DNA adducts in human cells.



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

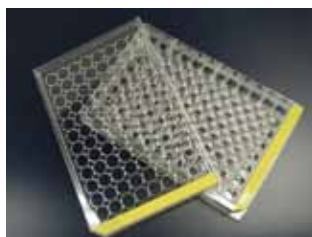
PROTAMINE SULFATE COATED ELISA PLATE

New

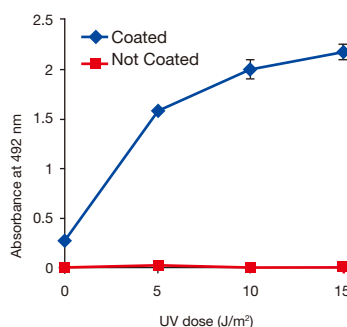
Protamine sulfate is a small cationic protein that binds to negatively charged DNA. Protamine sulfate coated wells capture sample DNA more efficiently; a critical step in the accurate and reproducible determination of DNA damage detection by ELISA.

- 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 96x5	CSR-NM-MA-P002	5x1 plate
PROTAMINE SULFATE COATED ELISA PLATE 96x10	CSR-NM-MA-P003	10x1 plate



Monoclonal Antibodies against DNA Damage

Antibodies against Nucleotide excision repair (NER) factors

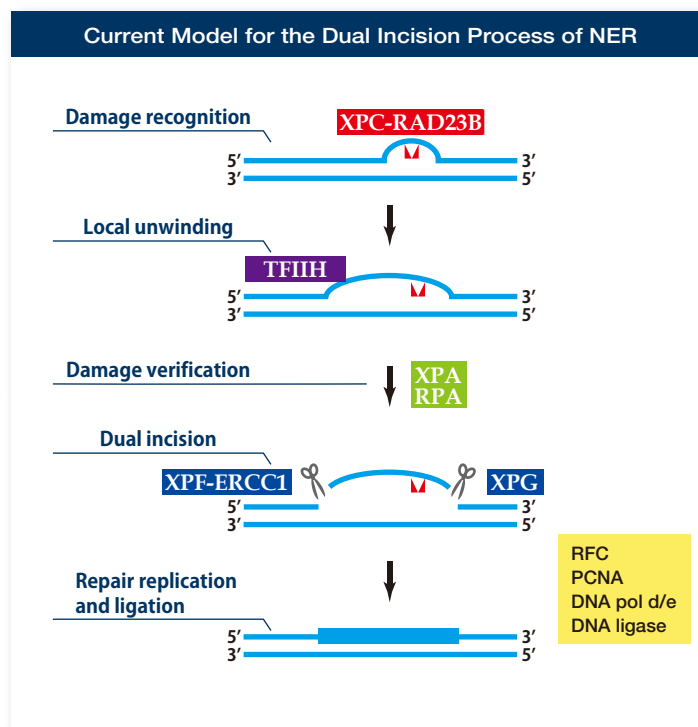
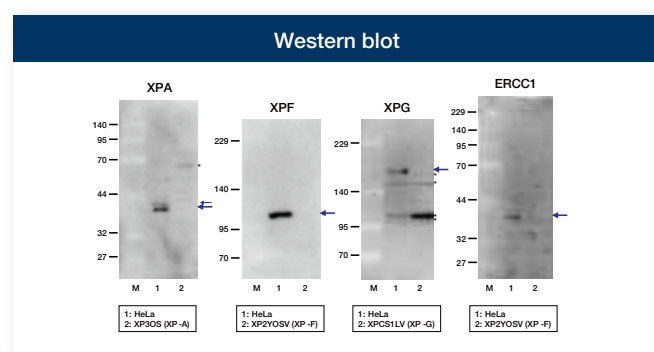
Anti XPA [Clone : A-2]

Anti XPG [Clone : G-26]

Anti XPF [Clone : 19-16]

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*.



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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DNA損傷検出 モノクローナル抗体

紫外線で誘起される DNA 損傷に特異的に結合します

紫外線誘発 DNA 損傷モノクローナル抗体

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

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

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

紫外線を浴びすぎると日焼け、光老化、皮膚がん、目の障害、免疫能の低下など、さまざまな悪影響が生じます。この健康影響に深く関係しているのが DNA 損傷です。紫外線照射により DNA のピリミジン塩基が連続した箇所に変化が生じ、3 種類の主要ピリミジン二量体（シクロブタン型ピリミジンダイマー、6-4 型光産物、Dewar 型光産物）が形成されます。これらの紫外線損傷は DNA の複製や転写に影響を与え、突然変異やアポトーシスなどを引き起こします。弊社抗体ブランド CAC では、これら 3 種類の紫外線 DNA 損傷をそれぞれ高特異的に認識するモノクローナル抗体を取りそろえました。ELISA による損傷定量や細胞および組織蛍光染色による損傷可視化に高性能を発揮し、DNA 修復、損傷応答、がん化、光老化、免疫、美容など幅広い研究分野において強力な研究ツールとなります。実際に、本抗体を用いた研究成果は、Nature や Cell など多くの主要国際雑誌に発表されています。

提供者：奈良県立医科大学先端医学研究機構ラジオアイソトープ実験施設 教授 森 俊雄 先生

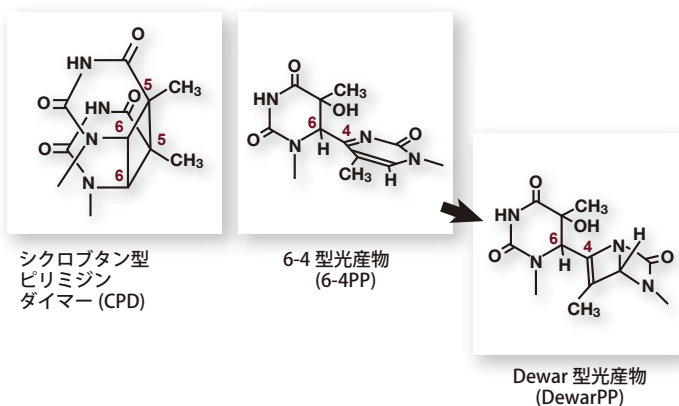
特長

- 各々の紫外線 DNA 損傷に特異的に反応
- ELISA、免疫蛍光法、免疫組織化学等のアプリケーションでご使用いただけます。
- DNA 損傷と修復の研究に最適です。
- DNA 損傷と修復のプロセスを可視化します。
- 癌研究、光生物学、皮膚科学、眼科学、免疫学、化粧品分野など幅広い研究分野でご使用いただけます。

参考文献

- (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).

太陽紫外線で誘発される主要 DNA 損傷



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品名	免疫動物	クローン	適用	品番	包装	希望販売価格
Anti CPDs	Mouse	TDM-2	ELISA / IC	NM-DND-001	1 vial	¥ 44,000
Anti 6-4PPs	Mouse	64M-2	ELISA / IC	NM-DND-002	1 vial	¥ 44,000
Anti DewarPPs	Mouse	DEM-1	ELISA / IC	NM-DND-003	1 vial	¥ 44,000

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CAC

検索



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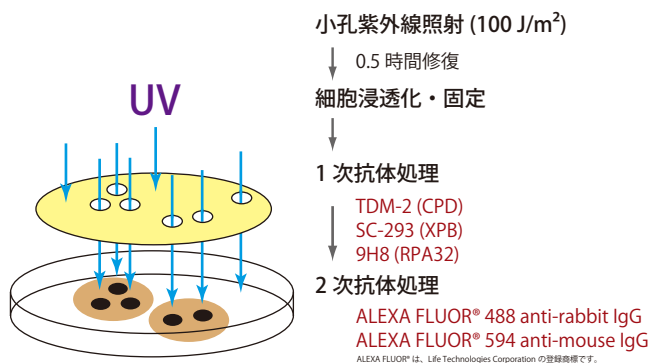
DNA損傷検出モノクローナル抗体

紫外線誘発 DNA 損傷モノクローナル抗体

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

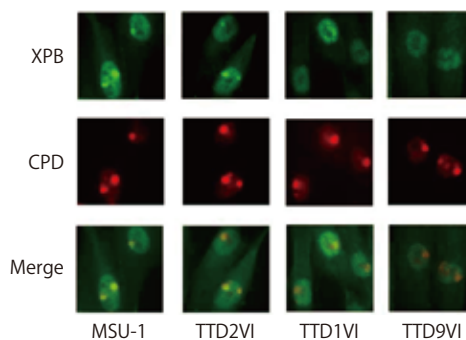
細胞免疫染色法 (immunocytochemistry)

小孔紫外線照射と蛍光免疫染色を利用した DNA 修復の可視化



DNA 損傷抗体は蛍光免疫染色に応用できるため、次のような実験が可能となる。ポリカーボネート製フィルターの小孔を利用して、細胞核の 1-3 ヶ所をスポット状に紫外線照射する。照射直後、あるいは修復後、細胞内の DNA 損傷や修復タンパク質を特異抗体を用いて二重に蛍光染色する。これらの蛍光画像を比較することにより、修復タンパク質の損傷部位への集積の有無や、複数の修復タンパク質の集積順序などの解析が可能となる。

紫外線局所照射後の XPB の損傷部位への集積

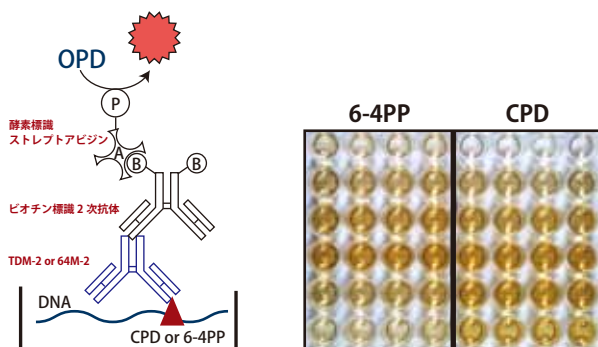


Nishiwaki et. al., *J. Invest. Dermatol.* 122: 526-532, 2004.

ヒト正常細胞 (MSU-1) では、紫外線照射 30 分後には、修復タンパク質 XPB は局所 DNA 損傷部位に集積し修復に関与していることがわかる。一方、修復欠損遺伝病 TTD (硫黄欠乏性毛髪發育異常症) 細胞では、損傷部位に集積する XPB は正常細胞に比べて少ないことがわかる。

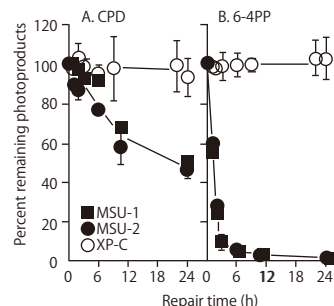
ELISA

ELISA 法による紫外線誘発 DNA 損傷の測定



DNA 損傷抗体を ELISA (酵素標識免疫法) に応用し、DNA 中の紫外線損傷を高感度に検出することができる。紫外線照射直後、あるいは修復後の細胞や組織からゲノム DNA を精製し、一定量を 96 プレートにコートする。DNA 損傷抗体を損傷に結合させた後、ビオチン標識 2 次抗体および酵素標識ストレプトアビジンでシグナルを増幅させる。最後に、基質を加えて 492 nm で測定する。

ELISA 法による DNA 損傷修復動態の解析



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

ELISA を用いた DNA 修復実験の結果を示す。ヒト正常細胞 (黒シンボル) は紫外線で誘発されたシクロブタン型ダイマー (CPD) の 50% を 24 時間で、また、(6-4) 光産物 (6-4PP) の 90% を 3 時間で修復する。これらの DNA 損傷はともにヌクレオチド除去修復で修復されるが、6-4PP は CPD に比べ二本鎖 DNA を大きく歪めるために優先的に修復される。一方、修復欠損遺伝病である色素性乾皮症 XP-C 細胞では両損傷のゲノム DNA からの修復は起こらない。

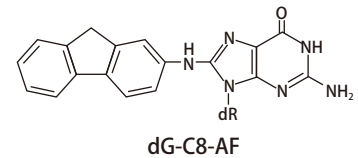
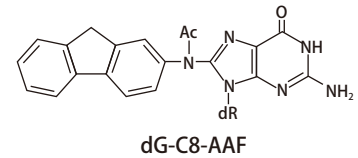
抗アセチルアミノフルオレン-DNA 付加体モノクローナル抗体

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

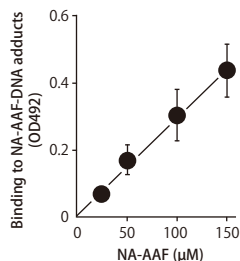
アセチルアミノフルオレン (2-AAF) は動物を用いた肝臓がん、膀胱がん、腎臓がん、大腸がん、および乳がんなどの発がん誘発実験に長く利用されてきました。2-AAF の活性体の一つである NA-AAF (N-acetoxy-2-AAF) は発がんに加え、細胞レベルで致死、染色体異常、突然変異などを引き起こします。こうした作用の原因として、DNA グアニン残基への結合体で 3 種類の AAF-DNA 付加体が考えられています。つまり、グアニン C8 位の付加体で dG-C8-AAF、その脱アセチル体の dG-C8-AF、および N2 位の付加体である dG-N2-AAF ですが、C8 位付加体の方が N2 位付加体に比べ形成量が多いです。AAF-1 抗体は 1 本鎖 DNA 中の dG-C8-AAF および dG-C8-AF と特異的に結合します。それ故、酵素標識免疫法 (ELISA) を用いて AAF-DNA 付加体を高感度検出できることに加え、蛍光免疫染色法を用いて細胞や組織中の付加体を可視化できます。こうした機能をもつ抗体は世界に例がなく、発がんや制がんに関する多くの研究に貢献することが期待されます。

提供者：奈良県立医科大学先端医学研究機構ラジオアイソトープ実験施設 教授 森 俊雄先生

AAF-1 抗体が結合する AAF-DNA 付加体

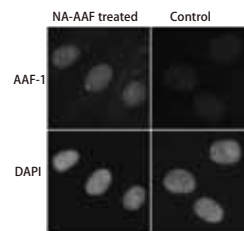


ヒト細胞における AAF-DNA 付加体の NA-AAF 処理濃度依存的形成



各種濃度の NA-AAF を細胞に 30 分間処理した後、DNA を抽出した。その後、AAF-1 (1/100) を利用した高感度 ELISA により AAF-DNA 付加体を測定した。

ヒト細胞における AAF-DNA 付加体形成の可視化



細胞に 200 μM NA-AAF あるいは対照溶媒を 30 分間処理した後、浸透化および固定を行った。その後、AAF-1 (1/25) および ALEXA FLUOR® 488 goat anti-mouse IgG を連続処理し、AAF-DNA 付加体を可視化した。細胞核 DNA は DAPI で染色した。

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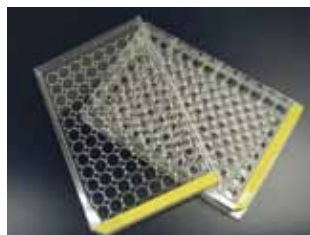
品名	免疫動物	クローン	適用	品番	包装	希望販売価格
Anti AAF-DNA adducts	Mouse	AAF-1	ELISA / IC	NM-MA-001	1 vial	¥ 50,000

DNA 損傷抗体での ELISA アッセイへ有用です

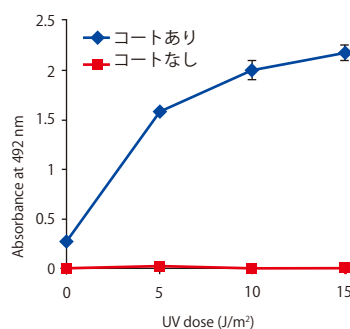
PROTAMINE SULFATE COATED ELISA PLATE

硫酸プロタミンはカチオン性タンパク質であり負電荷を持つ DNA と効率的に結合することが知られています。硫酸プロタミン処理は、プレートへの DNA 固相化を強固に安定化します。DNA 損傷抗体での ELISA アッセイでより安定した「正確なデータ」を得るために、本製品をご利用ください。

- 安定した DNA 結合性
- 少量 (低濃度) サンプルでも高いシグナルを検出
- 室温保存が可能
- プレートシール含む



硫酸プロタミンコートの有無による結合能の比較



UVC 照射後の DNA を 20 ng/well の濃度で固相化し、CPDs (Cyclobutane pyrimidine dimers) と特異的に結合する抗体 (Anti-CPDs [クローン：TDM-2]) を用いて ELISA 法により DNA 損傷量を定量した。硫酸プロタミンコートにより少量の DNA 量でも安定に保持することができ、より正確なデータを得ることが可能です。

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品名	品番	包装	希望販売価格
PROTAMINE SULFATE COATED ELISA PLATE 96	NM-MA-P001	1 plate	¥ 2,000
PROTAMINE SULFATE COATED ELISA PLATE 96×5	NM-MA-P002	5x1 plate	¥ 9,500
PROTAMINE SULFATE COATED ELISA PLATE 96×10	NM-MA-P003	10x1 plate	¥ 18,000



DNA損傷検出モノクローナル抗体

ヌクレオチド除去修復 NER 機構関連因子抗体

Anti XPA [Clone : A-2]

Anti XPF [Clone : 19-16]

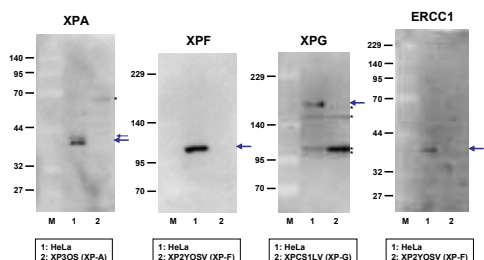
Anti XPG [Clone : G-26]

Anti ERCC1 [Clone : E1-44]

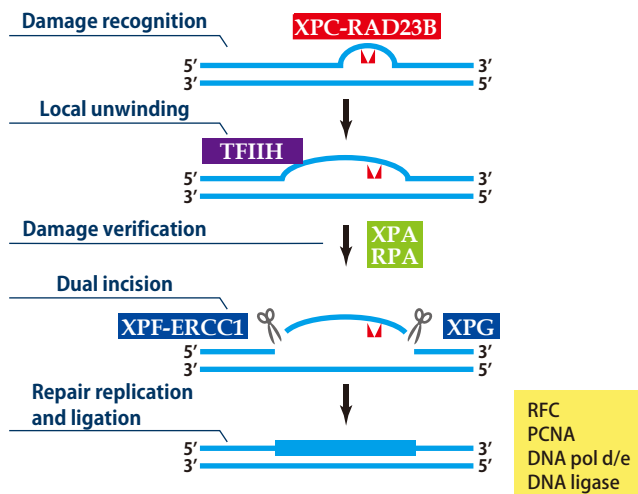
ヌクレオチド除去修復 (nucleotide excision repair: NER) は紫外線による DNA 傷害やかさ高い化学物質の塩基付加体など多種の DNA 傷害を修復できる重要な DNA 修復機構であり、NER 機構に異常をもつヒト遺伝疾患として色素性乾皮症 (xeroderma pigmentosum: XP) やコケイン症候群 (Cockayne syndrome: CS) 等が知られています。NER 機構のコア因子である XPA、XPF、XPG、ERCC1 に対する全ての抗体は、ウェスタンブロッティングへの適用が確認されています。

提供者：金沢大学医薬保健研究域薬学系 教授 松永 司 先生

Western blot 検出



Current Model for the Dual Incision Process of NER



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品名	免疫動物	クローン	適用	品番	包装	希望販売価格
Anti XPA	XPA タンパク質は 273 アミノ酸から成り、NER 反応に必須の因子である。このタンパク質に先天的異常をもつ色素性乾皮症 A 群患者は重篤な NER 欠損を示し、太陽露光部において著しい高発がん性を示す。XPA タンパク質は損傷 DNA に親和性があり、RPA、ERCC1、TFIIH、XAB1、XAB2 タンパク質など多くの NER 因子とも相互作用し、損傷 DNA 鎖の確認や修復複合体の足場として働くと考えられている。					
	Mouse	A-2	WB	KUP-TM-M01	100 µl	¥ 50,000
	Mouse	5F12	WB / ELISA	70-031*	50 µg	¥ 20,000
Anti XPF	Mouse	5F12	WB / ELISA	70-032*	250 µg	¥ 60,000
	Mouse	19-16	WB / IF	KUP-TM-M02	100 µl	¥ 50,000
Anti XPG	XPG は 1186 アミノ酸から成り、推定分子量は 133 kDa であるが、SDS-PAGE では 180 kDa 付近に泳動される。色素性乾皮症 G 群の責任因子であるが、変異の種類によってはコケイン症候群も併発する。XPG タンパク質は、DNA が 5' から 3' 方向に一本鎖から二本鎖になる境界でニックを入れる構造特異的エンドヌクレアーゼであり、XPF-ERCC1 複合体とは逆の極性をもつ。NER 機構では DNA 損傷の両側で切断が起こる Dual incision の 3' 側切断を担当する。					
	Mouse	G-26	WB	KUP-TM-M03	100 µl	¥ 50,000
Anti ERCC1	297 アミノ酸から成る ERCC1 タンパク質は XPF と安定複合体を形成し、これは相互の安定化にも寄与する。XPF-ERCC1 複合体は、DNA が 5' から 3' 方向に二本鎖から一本鎖になる境界でニックを入れる構造特異的エンドヌクレアーゼ活性をもち、NER 反応では DNA 損傷の両側で切断が起こる Dual incision の 5' 側切断を担当する。					
	Mouse	E1-44	WB	KUP-TM-M04	100 µl	¥ 50,000

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(11762)

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