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 dialysed against PBS. The dialysate was then lyophilized.

FORMULATION:

This antibody is lyophilized form.
Reconstitute with 100 µl of distrilled 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**.

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).

For research use only. Not for clinical diagnosis.

RELATED PRODUCTS:

NMDND001	Anti-cyclobutane pyrimidine dimers (TDM-2)
NMDND002	Anti-(6-4) photoproducts (64M-2)

TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
URL: http://www.cosmobio.co.jp e-mail: export@cosmobio.co.jp
[Outside Japan] Phone : +81-3-5632-9617 [国内連絡先] Phone : +81-3-5632-9610
FAX : +81-3-5632-9618 FAX : +81-3-5632-9619

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

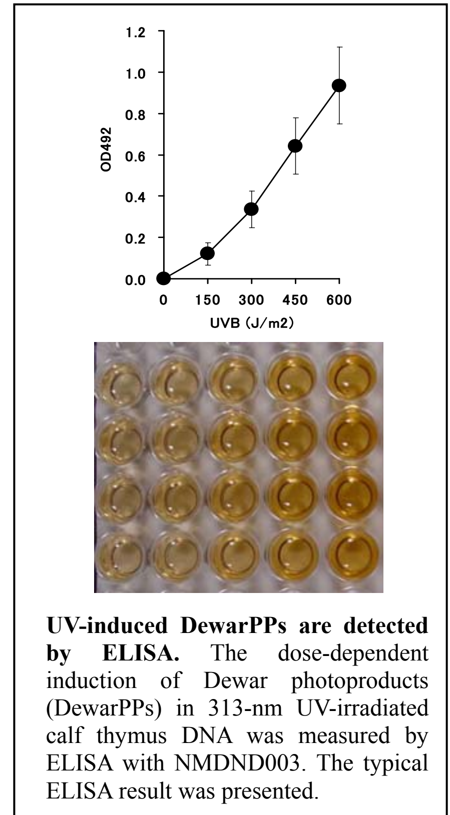
6) Plate cells in 10-cm dishes and culture one or two days.
7) Wash cells once by Dulbecco's PBS (DPBS) and irradiate cells with UVB (for example ; 0, 150, 300, 450 J/m^2 of 313 nm UV). To study DNA repair, following UV irradiation with 300 J/m^2, incubate cells for a variety of times (for example ; 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 6.0 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.



UV-induced DewarPPs are detected by ELISA. The dose-dependent induction of Dewar photoproducts (DewarPPs) in 313-nm UV-irradiated calf thymus DNA was measured by ELISA with NMDND003. The typical ELISA result was presented.

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 DEM-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 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, Na_2HPO_4 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, H_2O_2 (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 H_2SO_4 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 $2x10^5$ cells per dish, then incubate for one or two days in a CO_2 incubator.)
2) Wash cells once by DPBS and irradiate cells with UVB [for example ; 500 J/m^2 of 313 nm UV for whole cell irradiation (6)].

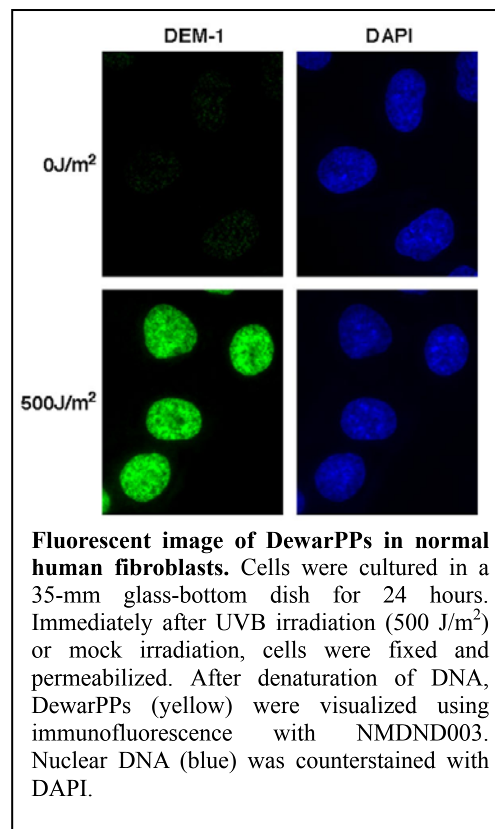
B. Cell fixation and permeabilization

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 DEM-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 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 DewarPPs in normal human fibroblasts. Cells were cultured in a 35-mm glass-bottom dish for 24 hours. Immediately after UVB irradiation (500 J/m^2) 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.

041206-1

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
URL: http://www.cosmobio.co.jp e-mail: export@cosmobio.co.jp
[Outside Japan] Phone : +81-3-5632-9617 [国内連絡先] Phone : +81-3-5632-9610
FAX : +81-3-5632-9618 FAX : +81-3-5632-9619

紫外線で誘起される DNA 損傷に特異的に結合します

DNA 損傷検出モノクローナル抗体

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

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で変化が生じ、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。コスモ・バイオ抗体ブランド 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免疫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。

特　長

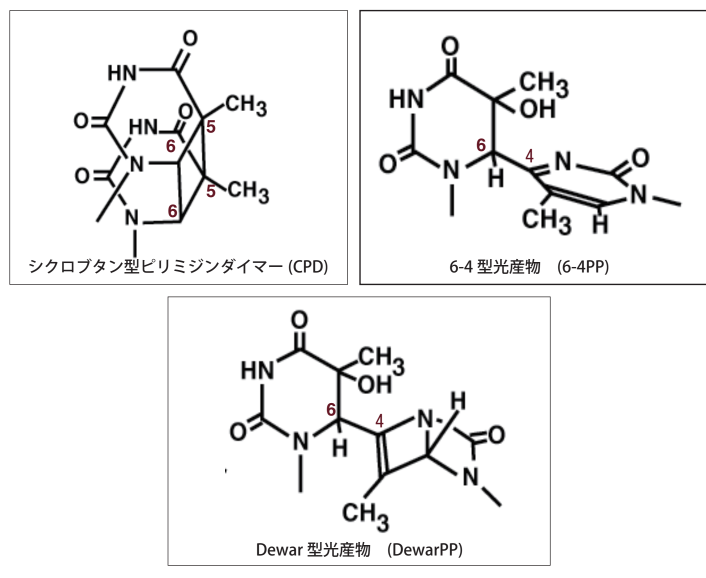
- 各々の紫外線 DNA 損傷に特異的に反応
- ELISA、免疫蛍光法、免疫組織化学等のアプリケーションでご使用いただけます。
- DNA 損傷と修復の研究に最適です。
- DNA 損傷と修復のプロセスを可視化します。
- 癌研究、光生物学、皮膚科学、眼科学、免疫学、化粧品分野など幅広い研究分野でご使用いただけます。

【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



コスモ・バイオ株式会社　メーカー略号：CAC

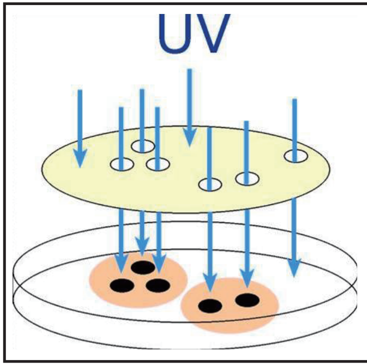
品名	免疫動物	クローン	適用	品番	包装	希望販売価格
Anti CPDs	Mouse	TDM-2	ELISA ／ IC ／ IHC	NM-DND-001	1VIAL	￥44,000
Anti 6-4PPs	Mouse	64M-2	ELISA ／ IC ／ IHC	NM-DND-002	1VIAL	￥44,000
Anti Dewar PPs	Mouse	DEM-1	ELISA ／ IC	NM-DND-003	1VIAL	￥44,000

人と科学のステキな未来へ
コスモ・バイオ株式会社

アプリケーション例

細胞免疫染色法（immunocytochemistry）

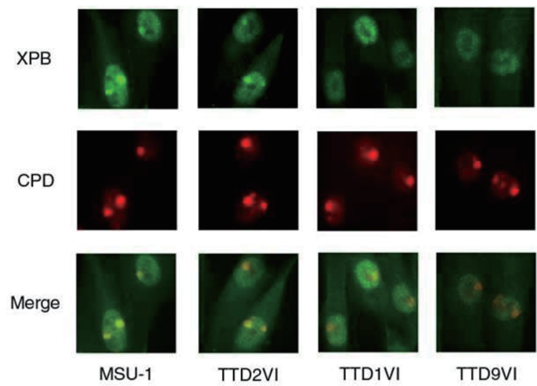
小孔紫外線照射と蛍光免疫染色を利用した DNA 修復の可視化



小孔紫外線照射 (100 J/m^2)
↓ 0.5 時間修復
細胞浸透化・固定
↓
1 次抗体処理
TDM-2 (CPD)
SC-293 (XPB)
9H8(RPA32)
↓
2 次抗体処理
Alexa 488 anti-rabbit IgG
Alexa 594 anti-mouse IgG

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 損傷や修復蛋白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、修復蛋白の損傷部位への集積の有無や、複数の修復蛋白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

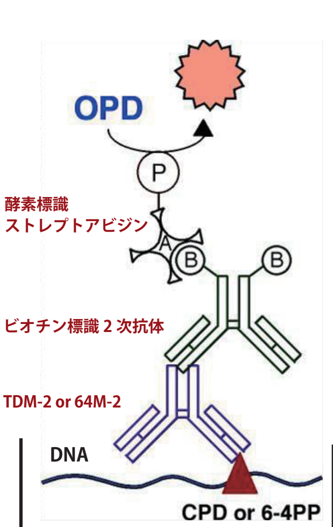


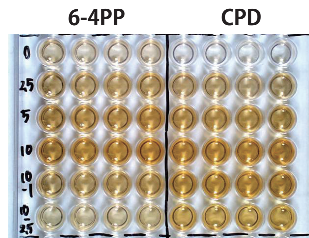
Nishiwaki et al., J. Invest. Dermatol. 122: 526-532, 2004.

ヒト正常細胞 (MSU-1) では、紫外線照射 30 分後には、修復蛋白 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（硫黄欠乏性毛髪発育異常症）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

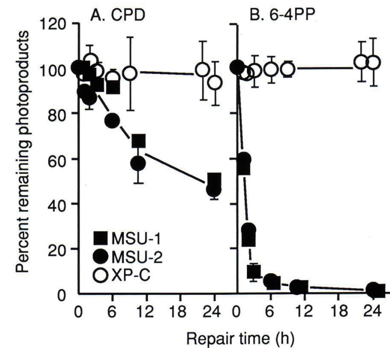
ELISA 法による紫外線誘発 DNA 損傷の測定





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、基質を加え着色させ 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 を大きく歪ませ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。

お手持ちの作製抗体を共同販売ブランドの CAC にエントリーしませんか

コスモ・バイオでは大学、研究機関由来の抗体製品化をお手伝いします
●市販品では入手困難な抗体を作製し、その成果を啓蒙したい
●所有の抗体を用いた研究が論文に掲載され、海外を含む他の研究者から問い合わせが寄せられている
●海外に抗体を配送したいが、輸送上の制約が多く対応できない
●R&D の一環で抗体を多数作製し、新たな有用性を模索したい

このような案件のある研究者の皆様は是非ご相談下さい。精製酵素、組み換えタンパク質等の試薬シーズをお持ちの方も歓迎いたします。

連絡先：コスモ・バイオ株式会社　開発部　担当：内田
TEL ／ FAX：03-5632-9605 ／ 03-5632-9614
e-mail：kaihatsu@cosmobio.co.jp

お願い および 注意事項

● 希望販売価格 ･･･「希望販売価格」は参考であり、販売店様からの販売価格ではございません。
記載の希望販売価格は２００８年４月１日現在の希望販売価格です。
予告なしに改定される場合がありますので、ご注文の際にご確認下さい。消費税は含まれておりません。
● 使 用 範 囲 ･･･記載の商品は全て、「研究用試薬」です。
人や動物の医療用・臨床診断用・食品用等としては使用しないよう、十分ご注意ください。

取扱店

人と科学のステキな未来へ
コスモ・バイオ株式会社

〒135-0016　東京都江東区東陽 2-2-20 東陽駅前ビル
URL : http://www.cosmobio.co.jp/
● 営業部（お問い合わせ）
TEL :（03）5632-9610　FAX :（03）5632-9619
TEL :（03）5632-9620