ELISA kit for measuring UV-induced DNA damage

High Sensitivity CPD/Cyclobutane Pyrimidine Dimer ELISA kit
(with mAb clone TDM-2)

Catalog Number : NM-MA-K001(96 tests)

For research use only, Not for diagnostic use.

- Please read this manual thoroughly before use -
INTRODUCTION

Prolonged exposure to solar UV radiation may result in harmful acute and chronic effects to the skin (including skin cancers), eye, and immune system. These harmful effects appear to be closely related to UV-induced 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 DNA strand. These helix-distorting DNA lesions are repaired exclusively by a nucleotide excision repair system in humans. To better study molecular events surrounding UV-induced DNA damage and repair, Mori et al. previously developed and characterized monoclonal antibody (mAb) specific for CPDs and mAb specific for 6-4PPs (1) while Matsunaga et al. developed and characterized mAb specific for DewarPPs (2). Three of these antibodies (CPDs: clone TDM-2; 6-4PPs: clone 64M-2; DewarPPs: clone DEM-1) continue to be cited frequently in the literature, often for use in ELISA by a recommended procedure.

This High Sensitivity Cyclobutane Pyrimidine Dimers (CPDs) ELISA Kit is the only commercially available ELISA utilizing anti-CPDs clone TDM-2 and has been optimized for high sensitivity detection of CPDs in DNA purified from cultured cells or from skin epidermis. This ELISA detects CPDs from dipyrimidines in all DNA sequence contexts (i.e., TT, TC, CT and CC). Thus, the availability and convenience of this ELISA Kit will contribute to further understanding molecular mechanisms involved in cellular responses to UV radiation and DNA damage with applications across many research fields including cancer research, photobiology, dermatology, ophthalmology, immunology, and cosmetics science.

Figure 1: Structures of UV-induced DNA damage in thymine-thymine sequence

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ASSAY PRINCIPLE

The format of this assay is ELISA with colorimetric detection. In brief, genomic DNA purified from UV-damaged cells is heat denatured and applied to microtiter wells pre-coated with protamine sulfate. CPD specific monoclonal antibody clone TDM-2 (Cosmo Bio Cat. No. CAC-NM-DND-001) is then added to each well for thirty minutes and unbound antibody is removed by rinsing. The amount of TDM-2 antibody remaining in each well is then measured by sequential treatment of wells with biotinylated 2nd antibody, streptavidin-peroxidase, and o-Phenylenediamine (OPD). The reaction between peroxidase, H₂O₂ and OPD produces a yellow orange color, the strength of which is generally proportional to the amount of TDM-2 antibody remaining bound to the plate. The color development reaction is stopped and the absorbance of each well at 492 nm is measured with a spectrophotometer.

REACTIVITY

1) Anti-CPDs monoclonal antibody clone TDM-2 recognizes CPDs on single-stranded DNA.
2) TDM-2 binds to CPDs formed each dipyrimidine sequence context (TT, TC, CT and CC).
3) TDM-2 stably binds to CPDs in DNA longer than eight bases.
4) TDM-2 binds to CPDs in UV-irradiated DNA purified from a wide range of sources the prokaryote and eukaryote irradiated with UV.

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## KIT COMPONENTS

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>ELISA plate precoated with protamine sulfate (12 x 8 well strips)</td>
<td>1 plate</td>
</tr>
<tr>
<td>Positive standard</td>
<td>1 vial (1 μg), lyophilized. Reconstitute with 100 μL purified water before use.</td>
</tr>
<tr>
<td>Calf thymus DNA, UVC irradiated (10 J/m²)</td>
<td></td>
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<tr>
<td>Negative standard</td>
<td>1 vial (1 μg), lyophilized. Reconstitute with 100 μL purified water before use.</td>
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<tr>
<td>Calf thymus DNA, not irradiated</td>
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<tr>
<td>Assay Diluent Concentrate (10X)</td>
<td>1 vial (10 mL)</td>
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<tr>
<td>Wash Buffer Concentrate (20X)</td>
<td>2 x 15 mL vials</td>
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<tr>
<td>Blocking Reagent Concentrate (50X)</td>
<td>2 vials, lyophilized. Reconstitute each vial with 200 μL purified water before use.</td>
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<tr>
<td>Anti-CPDs Monoclonal Antibody (clone TDM-2) (100X)</td>
<td>1 vial, lyophilized. Reconstitute with 150 μL purified water for a 100X working solution.</td>
</tr>
<tr>
<td>Biotinylated 2nd antibody (100X)</td>
<td>1 vial, lyophilized. Reconstitute with 150 μL purified water for a 100X working solution.</td>
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<tr>
<td>Streptavidin-peroxidase (100X)</td>
<td>1 vial, lyophilized. Reconstitute with 150 μL purified water for a 100X working solution.</td>
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<td>OPD Tablet (2mg)</td>
<td>2 tablets</td>
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<tr>
<td>OPD Diluent Concentrate (10X)</td>
<td>2 x 600 μL vials</td>
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<td>Stop Solution</td>
<td>1 x 12 mL vial</td>
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<td>Plate Cover Film</td>
<td>3 covers</td>
</tr>
<tr>
<td>Instruction Manual</td>
<td>1 manual</td>
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</table>

## MATERIALS TO BE SUPPLIED BY THE USERS

- DNA samples
- DNA Purification Kit (for sample preparation).
  Recomendd: QIAamp Blood Kit (QIAGEN, Cat. No. 51104 or 51106)
- 100 °C Heating Block
- Ice bath (Crush ice)
- Purified water
- 10 μL - 1000 μL adjustable single channel micropipetters and disposable tips
- 50 μL - 150 μL adjustable multichannel micropipetters and disposable tips
- Reservoir for Wash Solution
- 1.5 mL tubes (for diluting samples)
- 15 mL or 50 mL tubes (for dilutions)
- 37°C Incubator (non-humidified)
- Absorbance microplate reader capable of reading 492 nm.
- Vartex mixer
- Desktop centrifuge

[www.cosmobio.co.jp](http://www.cosmobio.co.jp)
STORAGE

Unopened kit: 4 °C
Opened kit:
- Reconstituted solutions: -20 °C
- Other reagents: 4 °C
- Protamine Sulfate Coated ELISA Plate: room temperature, protect from light

PREPARATION OF REAGENTS

1. **ELISA plate precoated with protamine sulfate**
   Bring to room temperature (18-25 °C) before use. Return unused wells to foil pouch.

2. **Positive and Negative CPD Standards**
   Standards are lyophilized. Reconstitute with 100 μL of purified water. The concentration of the standard solution is 10 μg/mL.

3. **Assay Diluent**
   Dilute 10 mL of Assay Diluent concentrate (10X) with 90 mL purified water to make 100 mL of Assay Diluent (1X).

4. **Wash Buffer**
   Dilute 15 mL of Wash Buffer concentrate (20X) with 285 mL purified water to make 300 mL of Wash Buffer (1X).

5. **Blocking Reagent**
   The Blocking Reagent is lyophilized. Reconstitute with 200 μL of purified water. Upon reconstitution, the solution is a 50X concentrate. Dilute 1:50 with Assay Diluent to prepare Blocking Reagent Working Solution.

6. **Anti-CPDs**
   The Anti-CPDs antibody is lyophilized. Reconstitute with 150 μL of purified water. Upon reconstitution, the Anti-CPDs solution is a 100X concentrate. Dilute 1:100 with Assay Diluent to prepare Anti-CPDs Working Solution.

7. **Biotinylated 2nd Antibody**
   This 2nd antibody is lyophilized. Reconstitute with 150 μL of purified water. Upon reconstitution, the Biotinylated 2nd Antibody solution is a 100X concentrate. Dilute 1:100 with Assay Diluent to prepare Biotinylated 2nd Antibody Working Solution.

8. **Streptavidin-peroxidase**
   This streptavidin-peroxidase conjugate is lyophilized. Reconstitute with 150 μL of purified water. Upon reconstitution, the solution is a 100X concentrate. Dilute 1:100 with Assay Diluent to prepare Streptavidin-Peroxidase Working Solution.

9. **OPD Diluent**
   OPD Diluent is provided as a 10X concentrate. Dilute 500 μL of OPD Diluent concentrate (10X) with 4.5 mL of purified water to prepare 5 mL of 1X OPD Diluent. Prepare immediately before use.

10. **OPD Substrate Solution**
    Dissolve one OPD tablet in 5 mL 1X OPD Diluent to make Working OPD Substrate Solution. Prepare immediately before use.

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ASSAY PROTOCOLS

A. Cell culture and UV irradiation
1. Plate cells in 10 cm dishes and culture for one or two days.
2. Wash cells once with Dulbecco’s PBS (DPBS) and irradiate with UV (e.g., 0, 2.5, 5, 7.5, 10 J/m² at 254 nm). To study DNA repair, irradiate cells with 10 J/m² and incubate for various amounts of time before harvesting (e.g., 1, 3, 8, 24 hours) to allow repair.
3. Wash cells with 10 mL DPBS. Harvest by scraping cells from dish. Centrifuge at 10,000xg for 15 seconds at 4 °C
4. Store cell pellets at –80 °C until ready for DNA isolation.

B. DNA isolation
5. Purify genomic DNA using a QIAamp Blood Kit (QIAGEN, Cat. No. 51104 or 51106) or similar. DNA concentrations are calculated by absorbance at 260 nm.

C. DNA sample coating to the ELISA plate precoated with protamine sulfate
6. Prepare sample DNA or CPDs DNA Standards solutions to a concentration of 0.4 μg/mL with 1X Assay Diluent. Denature DNA solutions by heating to 100°C for 10 minutes, then chill rapidly in an ice bath for 15 minutes.
7. Apply 50 μL/well of denatured DNA solution to the ELISA plate wells precoated with protamine sulfate (duplicates recommended) and dry completely overnight by incubation at 37 °C.

D. DNA damage detection
8. Wash the DNA-coated plates 5 times with 150 μL/well of 1X Wash Buffer.
9. Add 150 μL/well Blocking Reagent Working Solution to each well to prevent non-specific binding of antibody.
10. Incubate 30 minutes at 37 °C
11. Wash the plates 5 times with 150 μL/well of 1X Wash Buffer.
12. Add 100 μL/well of Anti-CPD Working Solution and incubate 30 minutes at 37 °C.
13. Wash the plates 5 times with 150 μL/well of 1X Wash Buffer.
14. Add 100 μL/well Biotinylated 2nd Antibody Working Solution and incubate 30 minutes at 37 °C.
15. Wash the plates 5 times with 150 μL/well of 1X Wash Buffer.
16. Add 100 μL/well of Streptavidin-Peroxidase Working Solution and incubate 30 minutes at 37 °C.
17. Wash the plates 5 times with 150 μL/well of 1X Wash Buffer.
18. Add 100 μL/well Working OPD Substrate Solution to each well and incubate 30 minutes at 37 °C.
19. Add 50 μL/well Stop Solution to each well to stop enzyme reaction.
20. Mix gently and immediately determine the absorbance at 492 nm of each well using a spectrophotometer.

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EXAMPLE OF RESULTS

Figure 3: UV-induced CPDs in DNA measured by ELISA

Figure 4: Formation and repair of UV-induced CPDs in human cells measured by ELISA

UVC radiation induces CPDs in DNA of HeLa cells in dose-dependent manner. The initial level of CPDs induced by 10 J/m² of UVC gradually decreases over time as CPDs are repaired, indicating the capacity of nucleotide excision repair in HeLa cells.
SELECTED REFERENCES


More than 200 papers using TDM-2 antibodies have been published so far.

RELATED PRODUCT

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<th>Cat#</th>
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<td>NM-DND-001</td>
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<tr>
<td>Anti (6-4) photoproducts (6-4PPs) Monoclonal Antibody (Clone: 64M-2)</td>
<td>CAC</td>
<td>NM-DND-002</td>
</tr>
<tr>
<td>Anti Dewar photoproducts (DewarPPs) Monoclonal Antibody (Clone: DEM-1)</td>
<td>CAC</td>
<td>NM-DND-003</td>
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Cosmo Bio Co., Ltd.
Inspiration for Life Science
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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

HS CPD ELISA kit Cat#: NM-MA-K001
High Sensitivity CPD ELISA Kit
— Cyclobutane Pyrimidine Dimer —
with mAb clone TDM-2

Cosmo Bio’s new Cyclobutane Pyrimidine Dimer (CPD) ELISA Kit uses the highly sensitive and specific monoclonal anti-CPD antibody clone TDM-2, established by Mori et al. TDM-2 is widely cited in the literature and is considered to be the gold standard antibody for CPD detection and quantification. Cosmo Bio’s CPD ELISA Kit is the first and only commercially available ELISA kit using TDM-2. All components are optimized for high sensitivity CPD detection from cultured cells or skin epidermis.

**Features**
- TDM-2 Monoclonal Antibody
- Recognizes CPD in every dipyrimidine sequence context
- High Sensitivity
- Positive and Negative CPD standards
- Protamine Sulfate coated plates for strong DNA binding
- 1 year shelf life for unopened kit (store at 4°C)

**Experiment Example**

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 to DNA damage CPDs 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.

<table>
<thead>
<tr>
<th>Description</th>
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<th>Quantity</th>
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<tr>
<td>High Sensitivity CPD ELISA Kit</td>
<td>CSR-NM-MA-K001</td>
<td>1 kit (96 test)</td>
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<tr>
<td>High Sensitivity 6-4PP ELISA Kit</td>
<td>CSR-NM-MA-K002</td>
<td>1 kit (96 test)</td>
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</table>

**Standard DNA:** DNA Controls, Negative (0 J/m², 1 vial) and Positive (2.5, 5, 7.5, 10 J/m², each 1 vial). This Kit include only Negative DNA (0 J/m²) and Positive DNA (10 J/m²). Standard DNA is also available separately.

<table>
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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. Matsunaga et al. have established and characterized monoclonal antibodies against DewarPPs. These antibodies enable one to quantitate photoproducts in DNA purified from cultured cells or from skin epidermis using an enzyme-linked immunosorbent assay (ELISA) and to visualize and measure photoproducts in DNA from cultured cells or skin samples 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 cosmetic science.

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 cosmeticology

Reference


More than 200 papers using these antibodies have been published so far.

Features Table

<table>
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<tr>
<th>Description</th>
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<td>64M-2</td>
<td>ELISA / IC</td>
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<td>1 vial</td>
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<tr>
<td>3 Anti DewarPPs</td>
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<td>DEM-1</td>
<td>ELISA / IC</td>
<td>CSR-NM-DND-003</td>
<td>1 vial</td>
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Useful for ELISA assays with DNA damage antibodies

PROTAMINE SULFATE COATED ELISA PLATE

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.

<table>
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<tr>
<th>Description</th>
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<tr>
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For research use only, Not for diagnostic use.
高感度紫外線誘発DNA損傷測定キット

High Sensitivity CPD ELISA kit
— Cyclobutane Pyrimidine Dimer —

太陽紫外線で最も誘発されるDNA損傷はシクロブタン型ピリミジンダイマー（CPDs）です。本キットは、CPDsの検出・定量用抗体のスタンダードとして長年にわたって世界中で使用されてきたCPDs特異的モノクローナル抗体（クローン：TDM-2）を初めて使用した高感度紫外線誘発DNA損傷CPD測定ELISAキットです。本キットは、紫外線に敏感な細胞応答、癌研究、光生物学、皮膚疾患、眼科、免疫学、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。

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 研究教授 森建雄 先生

特長
■ 世界標準のCPDs特異的モノクローナル抗体 クローン：TDM-2を使用
■ 全てのジピリミジン（TT, TC, CT, CC）配列のCPDsを検出
■ 高感度
■ 紫外線照射DNAおよび未照射DNAを同様
■ サンプルDNAを安定に結合させる硫酸プロトン付着プレートを同様
■ 有効期限：1年

実験例

紫外線照射直後、あるいは修復後の細胞からゲノムDNAを精製し、一定量を96プレートにコーティング。TDM-2抗体をDNAサンプル中のCPDsに結合させた後、さらにピリミジン2次抗体および酵素標識ストレプトアビシンを結合させ、シグナルを増幅させる。最後に、基質を加えCPDsに対する抗体結合量を492nmの吸光度で測定する。

ヒト細胞における紫外線によるシクロブタン型ピリミジンダイマー（CPD）の誘発とその修復。10J/m²の紫外線照射後の修復を調べた。

コスモ・バイオ株式会社

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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]

太陽紫外線を浴びすぎると日焼け、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。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 種類の主なピリミジン二重体（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。耐性抗体ブランド CAC（Cosmobio Antibody Collection）では、これらの 3 種類の紫外線 DNA 損傷をそれぞれ特異的に認識するモノクローナル抗体を用意しています。ELISA による損傷定量や細胞および組織皮膚光色による損傷可視化化を可能に、DNA 修復、損傷対応、がん化、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。本抗体を使った研究成果は、Nature や Cell など多くの主要国際誌に発表されています。

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ELISA 法で DNA 損傷を正確に測定する手助けとなるプレートです

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 固相化を簡易かつ安定化します。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。

品名 品番 包装 希望販売価格
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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

標準 DNA

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UVC irradiated DNA sample (0, 2.5, 5, 7.5, 10 J/m²) NM-MA-R010 1 set ¥ 30,000

お願いおよび注意事項

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