## 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

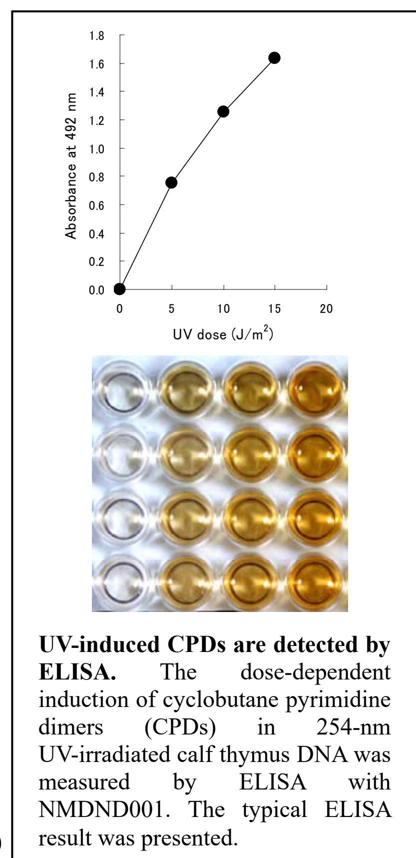
6) Plate cells in 10-cm dishes and culture one or two days.
7) Wash cells once by Dulbecco's PBS (DPBS) and irradiate cells with UV (for example ; 0, 5, 10, 15 J/$m^2$ of 254 nm UV). To study DNA repair, following UV irradiation with 15 J/$m^2$, incubate cells for a variety of times (for example ; 1, 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 0.2 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 CPDs are detected by ELISA.** The dose-dependent induction of cyclobutane pyrimidine dimers (CPDs) in 254-nm UV-irradiated calf thymus DNA was measured by ELISA with NMDND001. The typical ELISA result was presented.

E. DNA damage detection

14) Wash the DNA-coated plates 5 times with 150 µL/ well of 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 TDM-2 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.



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