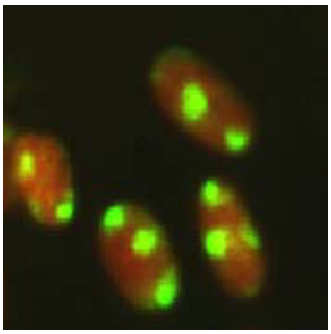
PROTOCOLS:
Immunofluorescence microscopy

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 UV [for example ; 10 J/m^2 of 254 nm UV for whole cell irradiation, or 100 J/m^2 of UV for local cell irradiation using a microfilter mask (1,5,6,9)].

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.)



Fluorescent image of localized CPDs in normal human fibroblasts. Cells were cultured in a 35-mm glass-bottom dish for 24 hours. Immediately after micropore UV irradiation (100 J/m^2), cells were fixed and permeabilized. After denaturation of DNA, CPDs (yellow) were visualized using immunofluorescence with NMDND001. Nuclear DNA (red) was counterstained with propidium iodide. A filter with 3-µm pores was used.

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 2 mL 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 TDM-2 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.

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